# Application of Comprehensive Proteomics to Map Metabolic Pathways of *Lactobacillus casei* under Carbohydrate Starvation and Growth under Low pH

By

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- Dr. Richard Wilson from the Centre Science Laboratory, University of Tasmania (Australia) contributed to data analysis.
- Dr. Anthony Baker from the Food Safety Centre, University of Tasmania (Australia), contributed to part of the experimental design of Chapter 3 during supervising a study undertaken by a Master of Applied Science student (coursework with experimental

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**TABLE OF CONTENTS**

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# ABSTRACT

Lactic acid bacteria, and more particularly lactobacilli, have been used for the production of fermented foods for centuries. They are amongst the most common microorganisms in the gastrointestinal tract of human and other animals; several lactobacilli have been recognised as probiotics due to their wide range of health promoting effects. One such probiotic species *Lactobacillus casei*, first described in cheese, is widely distributed in fermented food products and present within the human intestinal tract and oral cavity. *Lb. casei* strains have been recognized as having probiotic traits and have therefore been applied in numerous commercial food-based and drink-based health supplement products.

Survival of *Lactobacillus* species in harsh environments is one of the most important criteria for industrial and food-based applications. Environmental stresses affect the physiological properties, however, little is known about the molecular mechanisms underpinning survival of *Lb. casei* under stress conditions like those introduced during food fermentation, including changing composition of the food matrix, oxygen stress and acidity of the carrier food. To this end this thesis aims to provide information on the growth and physiological response of *Lb. casei* under nutritional stress (carbohydrate starvation) as well as growth under low pH conditions.

An initial study employed an integrated global proteomic response of the non-starter lactic acid bacteria *Lb. casei* strain GCRL163 under carbohydrate depletion to understand aspects of its survival following cessation of fermentation. This study built on prior published work from our group on lactose starvation in *Lb. casei* which, based on partial proteomic analysis and single-point analysis of end-products of metabolism, indicated that some enzymes involved in the glycolytic pathway were upregulated during lactose starvation and

that Tween 80 and other media constituents were potentially being utilized. The proteome of *Lb. casei* GCRL163 was analyzed quantitatively after growth in modified MRS (with and without Tween 80) with different levels of lactose (0% lactose, starvation; 0.2% lactose, growth limiting; 1% lactose, non-growth-limited control) using gel-free proteomics. Results revealed that carbohydrate starvation led to suppression of lactose and galactose catabolic pathways, which is consistent with the known mechanisms of induction of genes relating to lactose/galactose metabolism in the presence of the cognate substrate, as well as pathways for nucleotide and protein synthesis. Enzymes of the glycolysis/gluconeogenesis pathway, amino acid synthesis, and pyruvate and citrate metabolism became more abundant as well as other carbohydrate catabolic pathways, suggesting increased optimization of intermediary metabolism and scavenging alternative carbon and energy sources. This was consistent with temporal end-product analysis. Tween 80 did not affect growth yield; however, proteins related to fatty acid biosynthesis were repressed in the presence of Tween 80. These data suggested that *Lb. casei* adeptly switches to a scavenging mode, using both citrate and potentially Tween 80, and efficiently adjusts energetic requirements when carbohydrate starved and thus can sustain survival for weeks to months.

The presence of caproic and octanoic acids in culture fluids suggested that Tween 80 was being degraded during lactose starvation. However, the mMRS basal medium contained both citrate and acetate as possible carbon sources, so it was not clear whether Tween 80 was serving as a carbon source or whether it aided growth in the absence of lactose by eliminating biotin requirement, as indicated in the early literature (Williams *et al*., 1947). To investigate whether Tween 80 could be degraded as a carbon source, *Lb. casei* GCRL163 was cultured in mMRS with combinations of citrate and acetate, with and without Tween 80. Gel-free proteomic analysis was used to provide insight into the metabolic changes which occurred

when media either contained or lacked citrate and Tween 80. In the absence of carbohydrate, acetate and citrate as fermentable substrates, glycolysis and protein synthesis were strongly repressed, however moderate growth of GCRL163 was supported indicating that this strain has the ability to metabolise Tween 80. Growth could not be explained by release of oleic acid from Tween 80 following autoclaving and subsequent incubation of the medium at 30 $^{o}$C, as <6% of the Tween 80 was released as oleic acid and this did not change in controls over the growth period. The presence of Tween 80 strongly affected metabolic pathways related to sorbitol/sorbose, glycerol metabolism and fatty acid biosynthesis, up-regulating these pathways. The metabolism of Tween 80 appears to have a substantial effect on providing protection against acid shock and, based on the proteome, does so by strongly promoting fatty acid biosynthesis, protein hydrolysis and amino acid accumulation, and also stimulates compatible solute uptake and cell wall proteins. This response appears to be caused by Tween 80 causing cell membrane disturbances and generalised stress responses.

A further study was conducted to examine the growth of *Lb. casei* at low pH to simulate the acidic environments in food products which may affect the survival and thus the potential efficacy of probiotic bacteria. Acid stress adaptation responses of *Lb. casei* strains, including strain MJA12 which was isolated from the fermented milk product Yakult$^{TM}$ (which claims to contain the Shirota probiotic strain) and a genetically distinct Cheddar cheese isolate GCRL163, were investigated using gel-free, label-free proteomic analysis. The strains were grown under anaerobic conditions in MRS broth adjusted to and maintained at pH 4.5 or pH 6.5 in fermenters, with biomass collected during mid-exponential and early stationary growth phase. Approximately 35% of the proteome of each strain was identified to a >95% confidence level. Independent of growth phase, physiological adaptations to grow at low pH as predicted by the proteomic responses were different between the strains, however

both exhibited increased abundance in olipopeptide/dipepetide importers, peptidases/proteinases, branched chain aminotransferase, and the fatty acid biosynthetic pathway. These responses suggested exogenous amino acid accumulation, cell wall modulation and increased fatty acid unsaturation are the main means by which *Lb. casei* combats sudden acid stress, indicating that similar mechanisms are adopted during acid adaptation. Protein abundance data also revealed that acid adaptation varied between the strains in a growth phase-dependent manner, with strain MJA12 exhibiting most known acid adaptation-type responses when in the stationary growth phase, unlike strain GCRL163 which seems more directly responsive to acidic pH conditions earlier in growth. In addition, acid stress resulted in strain MJA12 (but not strain GCRL163) possessing increased levels of malolactic fermentation associated enzymes. The strain-dependent differences in the proteomes suggest adaptation of *Lb. casei* strains varies and thus strains could potentially react, behave and have different levels of persistence in either food or host systems.

Furthermore, the effect of growth under acidic conditions on the adhesion ability of *Lb. casei* cheese isolate GCRL163 and fermented milk isolate MJA12 was examined using HT-29 cells as an *in vitro* model for intestinal epithelium cells. The strains were grown under anaerobic conditions in MRS broth adjusted and maintained at pH 4.5 or pH 6.5 in fermenters, with biomass collected during early stationary growth phase. *Lb. casei* showed increased numbers of bacterial cells attaching to the cell line after adaptation to grow at pH 4.5 compared with cultures grown at pH 6.5. Gel-free proteomic analysis was used to understand the nature of these changes. Treatment with 5 M LiCl, with the goal of enriching surface-associated proteins, demonstrated that proteins enriched in these fractions consisted mainly of transmembrane proteins, membrane-associated proteins, extracellular secreted proteins and weakly enriched peptidoglycan related proteins, including various small

cytosolic proteins. Other proteins present in LiCl extracts included glycolytic proteins (glyceraldehydes-3-phosphate dehydrogenase, enolase and lactate dehydrogenase) and several proteins of unknown functionality, notably a highly enriched hydrolase. Exposing HT-29 cells to 10 µg of dialysed LiCl extract decreased subsequent binding of both *Lb. casei* strains, implicating the involvement of proteins in these extracts in binding.

Collectively, the results in this thesis increase the understanding of the physiological response of *Lb. casei* when grown under conditions that may be encountered in fermented foods and which pose specific stress conditions, namely carbohydrate limitation and acid stress. The study presents the functional analysis of proteins which also provide new insight into the metabolic pathways engaged by *Lb. casei* in dealing with food relevant stresses.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **µg** | **Microgram** |
| **µl** | **Microliter** |
| **µM** | **Micromolar** |
| **0% L** | **0% Lactose** |
| **0.2% L** | **0.2% Lactose** |
| **1% L** | **1% Lactose** |
| **2DE** | **Two Dimensional Electrophoresis** |
| **BSA** | **Bovine Serum Albumin** |
| **CFU** | **Colony Forming Unit** |
| **CSIRO** | **Commonwealth Scientific and Industrial Research Organisation** |
| **DNA** | **Deoxyribonucleic Acid** |
| **DTT** | **Dithiothreitol** |
| **EDTA** | **Ethylenediaminetetraacetic acid** |
| **FAO** | **Food and Agriculture Organization** |
| **GIT** | **Gut intestinal tract** |
| **HPLC** | **High-performance liquid chromatography** |
| **kDa** | **Kilodaltons** |
| **L** | ***Listeria*** |
| **l** | **Liter** |
| **LAB** | **Lactic Acid Bacteria** |
| **Lb** | ***Lactobacillus*** |
| **M** | **Molar** |
| **m/z** | **Mass / Charge ratio** |

| | |
|---|---|
| **mg** | **Milligram** |
| **min** | **Minute** |
| **ml** | **Milliliter** |
| **mM** | **Milimolar** |
| **MRS** | **de Man, Rogosa and Sharpe Medium** |
| **MW** | **Molecular Weight** |
| **nanoLC-MS/MS** | **Nanoscale liquid chromatography coupled to tandem mass spectrometry** |
| **NCBI** | **National Centre Biotechnology Information** |
| **NSLAB** | **Non-Starter Lactic Acid Bacteria** |
| **NTW** | **No Tween 80** |
| **OD** | **Optical Density** |
| **PCR** | **Polymerase Chain Reaction** |
| **rpm** | **Revolutions per minute** |
| **SDS** | **Sodium Dodecyl Sulfate** |
| **SDS-PAGE** | **Polyacrylamide gel electrophoresis** |
| **TEMED** | **N,N,N',N'-Tetramethylethylenediamine** |
| **TRIS** | **2-Amino-2-hydroxymethyl-propane-1,3-diol** |
| **TW** | **Tween 80** |
| **v/v** | **Volume per volume** |

# CHAPTER ONE

# LITERATURE REVIEW

## 1.1 Introduction

The human gastrointestinal tract (GIT) microbiota is a complex ecosystem comprising 200 to 600 bacterial species with the number of bacteria within the gut about 10 times greater than that of eukaryotic cells in the human body (Palmer *et al*., 2007). At birth, the human GIT is sterile, with bacteria colonizing the gut during the first feeding (Palmer *et al*., 2007). Following infancy, the composition of intestinal microbiota remains relatively constant (Knight and Girling, 2003).

The stomach and small intestinal contain relatively small numbers of bacteria. In healthy adults recovery of bacteria from the jejunum can be very low, with no detection reported in up to 33% of samples (Neish, 2009). In contrast, the microbiology of the ileum region represents a transition zone between the jejunum and the colon, and the bacterial count can be as high as $10^9$ CFU/ml in the ileum region and predominantly includes Gram-negative facultative anaerobes. On crossing into the colon, the bacterial population changes dramatically, with strictly anaerobic bacteria predominating due to the very low oxygen concentration in the colon (Neish, 2009; Boon *et al*., 2014).

Genome-based and analytical chemical technologies, including metabolomics, metagenomics and proteomics, have advanced our understanding of the colonic microbiota and has enabled characterization of colonic bacteria in both healthy and diseased people (Hattori and Taylor, 2009; Lepage *et al*., 2013). Techniques based on 16S rRNA gene sequences have already revealed that the diversity of the human microbiota is much greater

than previously thought, including many novel bacterial species (Eckburg *et al*., 2005; Kennedy *et al*., 2014). In any given region of the gut, the composition of the microbiota has demonstrated variation along its diameter with certain bacteria preferring adherence to the mucosal surface, while others predominate in the lumen. In humans, the composition of microbiota is influenced by age, diet, and use of antibiotics (Eckburg *et al*., 2005; Ley, 2014).

The GIT microbiota plays an important role in the physiology of their host. It influences a range of intestinal functions and plays a key role in nutrition, in maintaining the integrity of the epithelial barrier and in the improvement of mucosal immunity (Shanahan, 2002; Tomaro-Duchesneau *et al*., 2014). The relationship between the host's immune system and non-pathogenic constituents of the microbiota is important in protecting the host from colonization by pathogenic species. This may be due to intestinal bacteria producing a variety of substances, ranging from relatively nonspecific fatty acids and peroxides to highly specific bacteriocins, which can inhibit or kill other pathogenic bacteria (Quigley, 2010; Mazmanian and Lee, 2014).

Russian scientist, Elie Metchnikoff, first proposed that lactic bacteria in fermented milk could promote the development of healthy intestinal microbiota. Specifically, the Nobel-laureate developed a theory that lactic acid bacteria (LAB) of the phylum *Firmicutes* in the GIT could extend life by preventing putrefaction. Since that time, it has been known that some LAB and the high G+C-content Gram positive bifidobacteria (phylum *Actinobacteria*) are also found naturally within human and animal cavities, including the GIT. Common human-associated LAB species include *Lactobacillus acidophilus, Lactobacillus gasseri, Lactobacillus plantarum,* and *Bifidobacterium breve* (Tannock, 1999). LAB are considered to be important components of the normal intestinal microbiota, which contribute to a variety of

functions including intestinal integrity, immune modulation, and pathogen resistance. Selected *Lactobacillus* and *Bifidobacterium* species are used widely as probiotics primarily in fermented food, dairy products and dietary supplements (Reid, 1999; Tannock, 1999).

Lactobacilli are distributed in various ecological niches throughout the GIT and constitute an important part of the indigenous microbiota of man and higher animals (Sharma and Devi, 2014). Their abundant is affected by several environmental factors, which include the level of oxygen, the presence or absence of specific growth substrates, pH, presence of secretions and bacterial interactions. They are infrequently associated with cases of gastrointestinal infection and strains employed technologically are regarded as non-pathogenic and safe microorganisms (Deepika and Charalampopoulos, 2010).

Lactobacilli appear to be a relatively minor component of human adult faecal microbiota, i.e. around 0.01% to 0.6% of total bacterial counts (Sghir *et al*., 2000; Dal Bello *et al*., 2003) *Lb. reuteri*, *Lb. salivarius*, *Lb. gasseri*, *Lb. ruminis*, and *Lb. crispatus* appear to be the preponderant indigenous *Lactobacillus* species (Lebeer *et al*., 2008). *Lb. fermentum*, *Lb. acidophilus*, *Lb. casei*, *Lb. johnsonii*, *Lb. brevis*, *Lb. rhamnosus*, *Lb. delbrueckii*, and *Lb. plantarum* can also be found in the human GIT at varying levels (Heilig *et al*., 2002). In comparison to the adult microbiota, the infant microbiota is highly unstable but also contains lactobacilli in variable numbers. The number of *Lactobacillus* cells in newborn was found to be in the range of $10^5$ CFU/g in faeces, while in infants one month and older, the counts ranged from $10^6$ to $10^8$ CFU/g.

Within human hosts, *Lactobacillus* species possess extensively documented effects including immunostimulation and reduction of the intensity and duration of diarrhoea

(Wolvers *et al*., 2010; Shao *et al*., 2014). In addition, many studies have reported beneficial effects of *Lactobacillus* against pathogenic microorganisms including bacteria, fungi and viruses. The ability to attach to GIT epithelium cells and to mucins has been recognized and it is one of a series of important characteristics that enhances intestinal persistence and allows antagonistic competition with pathogens, especially at the point of initial contact with the mucosa.

In the early twentieth century, the industrialization and commercialization of fermented food necessitated the need to turn traditional fermentation processes into controlled and rationalized food processing. Moreover, the food and beverage industry is rediscovering fermentation as a crucial step in product innovation (Steensels and Verstrepen, 2014). With this aim, fermentation research in food microbiology has focused on the isolation and phenotypic characterization of microbes originating from natural sources, such as spontaneous fermentation. This approach has delivered defined single and mixed-strain cultures suitable for industrial applications (Adamberg *et al*., 2014). However, the production of selected dairy starter cultures has been met with several obstacles, such as how different strains cope with limitations in carbon and nitrogen source availability, salt stress during brining, temperature fluctuations, pH changes, and phage predation during cheese fermentation (Champagne *et al*., 2005). Sensitivity to those factors and reaction to these can be essential for proper flavour formation and product quality. The robustness of the starter culture during cheese fermentation is enhanced by the presence of a rich consortium of microbes. Diversity in the starter culture is determined by different phenotypic characteristics linked to the coexistence of different genomic lineages of microbes. The genetic diversity present in wild strains includes a variety of features that are relevant to the improvement of

starter cultures (Leroy and De Vuyst, 2004; Akçelik *et al*., 2014), and this diversity is selectively propagated as a result of adaptation by the microbes to dynamic environments.

Lactobacilli are distributed in various ecological niches throughout the GIT and constitute an important part of the indigenous microbiota of man and higher animals (Sharma and Devi, 2014). Their abundance is affected by several environmental factors, which include the level of oxygen, the presence or absence of specific growth substrates, pH, presence of secretions and bacterial interactions.

Molecular-based research that concentrates on *Lactobacillus* has two important objectives: defining the circumstances driving the best performance of the *Lactobacillus* strains and allowing the selection of *Lactobacillus* strains with a well-defined molecular basis for functionality. These objectives are important in two contexts: firstly, selecting strains that are able to adapt optimally to niches that they temporarily encounter in the host and, secondly, in determining factors that directly contribute to health promotion effects. In general, the continuing increase in interest in probiotic bacteria has led to an increase in manufacture of functional foods and non-food products (such as dietary supplements) containing these bacteria.

In this literature review, the focus will be on probiotic strains from the genus *Lactobacillus*, given their long history of use in fermented milk, meat and vegetables and emerging importance in health promotion.

## 1.2 Lactobacilli as Probiotics and Health-Promotion

The Food and Agriculture Organization (FAO) and World Health Organization (WHO) define probiotics as "*live microorganisms which, when administrated in adequate amounts, confers a health benefit on the host*" (FAO/WHO, 2001). Probably the most studied probiotics include lactobacilli and bifidobacteria. These bacterial groups have a proven safety record both within the fermented foods industry, where they have been used for several years, and, more recently, in probiotic-supplemented foods. Probiotic therapy has been investigated for its effectiveness against a range of gastrointestinal diseases and disorders. These days several hundred commercial products contain probiotic formulations and are functionally associated with bacteria known for their role in manufacturing fermented foods (Klaenhammer *et al*., 2005; Sidira *et al*., 2014). The most commonly bacterial strains used in food products belong mainly to a heterogeneous group of species in the genera *Lactobacillus* and *Bifidobacterium* (Poolman, 2002).

Members of the genus *Lactobacillus* are aerotolerant, anaerobic, strictly fermentative, non-spore-forming, Gram-positive bacteria that belongs to the phylum *Firmicutes* (Claesson *et al*., 2007). Their main end-product of carbohydrate metabolism is lactic acid. Because of their metabolism, lactobacilli are the amongst the dominant bacteria in the proximal small intestinal e.g. duodenum and jejunum, where readily-available carbohydrate-containing substrates derived from the host's diet are used as primary nutrient sources (Bongaerts and Severijnen, 2001).

The genus *Lactobacillus* contains 171 described species as of April 2014 (http://www.bacterio.net/) and are generally regarded as safe due to their long associated use in fermented foods and their presence in the small and large intestine (Tuohy *et al*., 2003).

The beneficial nature of *Lactobacillus* strains is likely to be multifunctional and from existing evidence appears to be strain specific (Sharma and Devi, 2014). Colonization capability or direct inhibitory effects against pathogens are likely to be important factors in situations where probiotics have reduced the incidence and duration of gastroenteritis. *Lactobacillus* strains have been shown to inhibit pathogenic bacteria both *in vitro* and *in vivo* through several different mechanisms. These include production of antimicrobial peptides (bacteriocins), reduction of pH through production of short chain fatty acids, competition for nutrients, competition for adhesion sites on the gut wall and immune system modulation (Steer *et al*., 2000; Fooks and Gibson, 2002).

### 1.2.1 Probiotic and the Host

Current research has focused on understanding the relationship between probiotic bacteria and the gut microbiota and the human host, as a means of understanding the mechanisms of probiotic functionality that contribute to their beneficial features. The stomach and small intestine are colonised by few species of microbiota and are present due to attachment to epithelium cells or are in transit. The absence of bacteria in the upper tract seems to be due to the composition of the luminal medium (pancreatic secretion, acid, bile), which kills most ingested microorganisms. By contrast, the colon contains a complex and dynamic microbial ecosystem with high densities of living bacteria, which achieve concentrations of up to $10^{13}$ to $10^{14}$ CFU/g of luminal contents (Knight and Girling, 2003; De Preter *et al*., 2011). Several hundred grams of microbiota living within the colonic lumen interact with the host regularly. Some of these community members are potentially pathogenic and, if unchecked, can be a source of infection. However, the constant interaction between the human host and microbial guests can infer important health benefits.

Colonic microbiota carries out metabolic, trophic, and protective functions. A major metabolic function of colonic microbiota is fermentation of non-digestible dietary residues and the endogenous mucus produced by the epithelium (Roberfroid *et al*., 1995). Owing to the diversity of the microbial community many enzymes and biochemical pathways that are distinct from the host's own resources are employed for these purposes. Overall, the outcome of these complex metabolic activities comprises improvement of metabolic energy and absorbable substrates for the host, coupled with supply of energy and nutritive products for bacterial growth and proliferation. In the right colon and caecum, fermentation is intense with rapid creation of short-chain fatty acids, an acidic pH (4.8 – 6.0), and rapid bacteria growth (Cummings *et al*., 1987). By contrast, utilisable substrates in the left or distal colon are less available and thus the pH is close to neutrality, putrefactive processes become quantitatively more important, and bacterial populations are relatively static. Colonic microorganisms also have roles in vitamin synthesis and in absorption of calcium, magnesium, and iron (Younes *et al*., 2001).

Trophic function comprises two major roles: epithelial cell growth and differentiation. The most important role of short chain fatty acids on colonic physiology is their trophic effect on the intestinal epithelium. Differentiation of epithelium cells is also greatly affected by interaction with resident microorganisms (Guarner and Malagelada, 2003). The proportion of production of crypt cells is decreased in the colon of rats bred in germ-free environments. Rat GIT crypts contain fewer cells compared with those of rats colonised by normal biota, suggesting that inter-luminal bacteria affect cell proliferation in the colon (Alam *et al*., 1994). The second role comprises interaction between gut bacteria and host immunity. The gut mucosa is the main layer between the immune system and the external environment (Knight and Girling, 2003). The relation between the host and microbiota at the mucosal interface

plays an important role in development of a competent immune system. Bacterial colonisation of the GIT affects the structure of gut-associated lymphoid tissue (Helgeland *et al*., 1996). How probiotic influence the immune system, more details discussed in section 1.3.3.

Indigenous bacteria with protective functions work as a crucial "wall of resistance" impeding colonisation by exogenous microbes and, therefore, are relevant in prevention of invasion of tissues by pathogens (Taguchi *et al*., 2002). Colonisation resistance also applies to opportunistic bacteria that are present in the gut but have restricted growth. The evenness between species of resident bacteria assists in stability in the microbial population within the same individual under normal conditions. Several mechanisms have been involved in the barrier effect. *In vitro*, bacteria compete for adherence sites in the brush border of intestinal epithelial cells. Adherent probiotic bacteria can prevent attachment and subsequent invasion of epithelial cells by pathogens like *Escherichia coli* and *Listeria monocytogenes* (Bernet *et al*., 1994). Some ingested probiotics can affect the composition and behaviour of intestinal microbiota and it is of interest to explore factors that determine the survival of probiotics while in GIT transit (O'Flaherty and Klaenhammer, 2010).

Bacteriocins within *in vitro* studies showed inhibition of pathogens, a similar response was mediated by low molecular weight substances such as hydrogen peroxide and short chain fatty acids such as lactic acid. Bacteriocins produced by lactobacilli can be classified into three groups: i) class I lantibiotics, harbouring unusual amino acids such as lanthionine; ii) class II, heat stable non-lantibiotics; and iii) class IV, cyclic antimicrobial peptides (Maqueda *et al*., 2008). The class II bacteriocin Abp118 produced by *Lb. salivarius* strain UCC118 is able to protect mice against infection by pathogens especially, *L. monocytogenes* (Corr *et al*.,

2007). Additionally, probiotic strains may also produce other types of antimicrobial compounds. For example, reuterin produced by *Lb. reuteri* ATCC 55730 consists of an equilibrium of 3-hydroxypropionaldehyde with its hydrated, and dimeric, derivatives (Maqueda *et al*., 2008). This antimicrobial shows inhibition activity not only against Gram positive and Gram negative bacteria but is also active against yeast, fungi, protozoa and viruses. Probiotic bacteria can also form deconjugated bile acids, which are derivatives of normal bile salts. Deconjugated bile acids show a stronger antimicrobial activity compared to the bile salts synthesized by the host organisms (Maqueda *et al*., 2008).

Probiotics can also influence the composition and behaviour of intestinal microbiota *via* an anti-adhesion activity. Since probiotics have the ability to adhere to epithelial cells in tissue culture assays they may be able to actively block adherence of competing microbes. This mechanism is important for probiotic activity within the host. The anti-adherence effect might be the result of competition between probiotic and pathogen for the receptor or the induction of increased mucin production by the probiotic strain Oelschlaeger *et al*., (2010). Mack *et al*., (2003) has mentioned in his work that induction of MUC3 mucin in HT-29-MTX cells when co-cultured with *Lb. plantarum* 299v or *Lb. rhamnosus* GG MUC3 mucin subsequently inhibited the adhesion of pathogenic *E. coli* strain E2348/69. However, the ability to inhibit the pathogen adhesion to human mucus appears to depend on both the specific probiotic and the pathogen strains. In addition, some commercial probiotic strains actually increased adhesion of *E. coli, L. monocytogenes* and *Salmonella* Typhimurium to human mucus (Maqueda *et al*., 2008).

### 1.2.2 *Lactobacillus* Adhesion Properties

Arguably the largest and most important interaction between organism microbe and its environment occurs at the cell surface (Saarela *et al*., 2000; Tiittanen *et al*., 2013). Cell surface properties precisely determine the ability of the microorganisms to adhere to both the intestinal mucus and to enterocytes (epithelial cells) (Jensen *et al*., 2014) (Table 1). The adhesion of probiotics to human mucosal cells has been suggested as an important prerequisite for probiotic action, the adhesion of probiotic cells is likely to aid longer persistence in the intestinal tract, stabilize the intestinal mucosal barrier, provide competitive exclusion of pathogens, and induce immunomodulation (Saarela *et al*., 2000; Larsen *et al*., 2007).

Studying the mechanisms of probiotic adhesion has been facilitated by *in vitro* experiments using cultured epithelium-associated cells and *in vivo* using human or animal colon biopsy samples, which are then examined by electron microscopy. To obtain a probiotic or pathogenic effect depending on the invasive bacteria species concentration, the bacteria must adhere to the cell surface of the digestive tract and two conditions must be present to allow the implantation of a bacterium: it must be able to multiply, depending on the presence of the nutrients, and it must be able to reside *in situ, i.e.*, adhere to the host cells and/or mucus.

Probiotic bacteria exhibit several surface determinants that are associated in their interaction with intestinal epithelial cells (Servin and Coconnier, 2003). The microbial adhesion process includes passive forces, electrostatic and hydrophobic interactions, lipoteichoic acids, and specific structures, such as external appendages covered by lectins (Servin and Coconnier, 2003). A variety of *in vitro* model systems have been used for routine

adhesion experiments. The most popular cell lines include HT-29 or Caco-2 human-derived adenocarcinoma cells (Velez *et al*., 2007; Dhanani and Bagchi, 2013) and immobilized intestinal mucus (Roos and Jonsson, 2002; Vesterlund *et al*., 2005).

**Table 1:** *Lactobacillus* strains adhere to mucus and enterocytes (epithelium cell) with different proposed mechanisms.

| Bacterium | Substratum | Concentration (CFU/ml) | Type of Adhesion | Adhesion condition | Reference |
|---|---|---|---|---|---|
| *Lactobacillus rhamnosus* | porcine colonic mucin (PCM), fibronectin, laminin, collagen IV | ~$10^7$ | Extracellular proteins | 1 h, 0.01% Triton X-100 in DMEM, 5% $CO_2$ and 95% air | Nishiyama *et al*., 2014 |
| *Lactobacillus plantarum* | Caco-2 cell lines | $10^9$ | Surface proteins | 2h, 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air | Li *et al*., 2015 |
| *Lactobacillus kefiri* | type III porcine gastric mucin | $10^8$ | Surface proteins | 2 h at 37 °C | Carasi *et al*., 2014 |
| *Lactobacillus rhamnosus* DR20<br><br>*Lactobacillus acidophilus* HN017 | HT-29, Caco-2, and HT29-MTX | $10^8$ | Unknown | 1 h or 2 h at 37°C in 10% $CO_2$ –90% air. | Gopal *et al*., 2001 |
| *Lactobacillus casei* strains | Mucin, fibronectin (pig plasma), fibronectin (human plasma) and collagen type I | $10^6$ | Mub and Msa covalently attached to the cell surface by a sortase-dependent mechanism | 4 h or 2 h at 37°C in 5% $CO_2$ –95% air. | Muñoz-Provencio *et al*., 2009 |

| | | | | | |
|---|---|---|---|---|---|
| *Lactobacillus reuteri* strains | Mouse colonic mucus (MCM) | $10^6$ | Unknown | 1 h incubation at room temperature | MacKenzie *et al*., 2010 |
| *Lactobacillus rhamnosus* Lcr35 | Caco-2 and HT29-MTX | ~$10^7$ | Pilus-specific Protein, cell surface proteins | 3 h at 37 °C under 5 % $CO_2$ | Nivoliez *et al*., 2015 |
| *Lactobacillus acidophilus* BG2FO4 | Caco-2, HT-29 and HT29-MTX cells | 2 x $10^8$ | Complexes containing lipoteichoic acid are involved in the adhesion of urogenital *Lactobacillus* | 1 h 37°C in 10% $CO_2$-90% air | Coconnier *et al*., 1992 |
| *Lactobacillus johnsonii* strains | Caco-2 | $10^8$ | Cell surface-associated lipoteichoic Acid | 1 h 37°C | Granato *et al*., 1999 |
| *Lactobacillus* strains | Caco-2 | ~$10^7$ | Surface proteins | 1.5 h 37°C | Tuomola *et al*., 1998 |
| *Lactobacillus crispatus* | HeLa cell line | ~$10^7$ | S-layer proteins | 1 h at 37 °C in 5% CO2 | Chen *et al*., 2007 |

Intestinal epithelial cells that play a principal role in the cross talk between the host and luminal content (Velez *et al*., 2007). In despite of many *in vitro* studies attempting to authenticate the adherence of *Lactobacillus* strains to epithelial surfaces, only a few *in vivo* (biopsy sample) studies have confirmed this adhesion (Valeur *et al*., 2004). *Lactobacillus* adhesion sites can be different depend on their target in the intestinal mucosa *e.g.* mucus components, extracellular material, according to their localization in the bacterial surface, *e.g*. surface layer proteins, and/or according to the way they are anchored to the bacteria surface, *e.g.* sortase-dependent proteins (Velez *et al*., 2007; Mangiapane *et al*., 2013).

The mechanisms of adhesion of *Lactobacillus* species to components of the intestinal mucosa, including information on the surface molecules mediating this adhesion and their corresponding receptors, are still fragmentary (Velez *et al*., 2007). In addition, and probably more essentially, the augmentation of adhesive surface compounds by the physicochemical properties of bacteria surfaces is still not clear in many cases, and perhaps depends on the type of compounds present, their structure and conformation. These characteristic are likely influenced by the physiological state of the cells (Deepika and Charalampopoulos, 2010).

The ability of *Lactobacillus* species to adhere to the GIT has been suggested to influence their interaction with host cells, by affecting the local microbial composition and by stimulating the immune system (Candela *et al*., 2008; Qin *et al*., 2009; Vintiñi and Medina, 2014). The adhesive abilities of *Lactobacillus* have been linked with their surface properties, which in turn are influenced by the composition, structure and organization of the cell wall. This includes the nature of the structural components of the cell surface, including lipoteichoic acids, polysaccharides, and cell surface proteins, all of which contribute to the net physicochemical properties of the bacteria surface, such as electrostatic charge and

hydrophobicity (Schar-Zammaretti and Ubbink, 2003). In addition, using a variety of model systems, such as epithelium cells, mucus components, and extracellular matrices, these surface components have been used to study adhesion capacity of *Lactobacillus* cells (Granato *et al*., 1999; Buck *et al*., 2005; Velez *et al*., 2007; van Baarlen *et al*., 2013).

The functional role of S-layer proteins is not yet clear. Surface S-layers are crystalline arrays of proteinaceous subunits present as the outmost layer of the cell wall in several species of the genus *Lactobacillus*, as well as in many other bacteria (Hynönen *et al*., 2014). Despite the high similarity of the amino acid composition of all known S-layer proteins, the overall sequence similarity is, however, surprisingly small even between the *Lactobacillus* S-layer proteins (Avall-Jaaskelainen and Palva, 2005). Many studies have shown they mediate adhesion to host cell surfaces (Zhang *et al*., 2013; Carasi *et al*., 2014) . The S-layer of diverse strains of *Lb. acidophilus* has been proposed to mediate adhesion to porcine epithelial cells (Kos *et al*., 2003; Zhang *et al*., 2013) and mouse epithelial cells of other animals (Schneitz *et al*., 1993; Frece *et al*., 2005). The S-layer of *Lb. brevis* ATCC strain has been shown to be accountable for the interaction with extracellular matrix components, such as fibronectin, laminin, and collagen (de Leeuw *et al*., 2006). Furthermore, the S-layer protein CbsA of *Lb. crispatus* JCM 5810 has been shown to mediate adhesion to laminin and collagen ( Martinez *et al*., 2000; Antikainen *et al*., 2002).

Sanchez *et al*. (2008) compared six methods for extracting the surface-associated proteins of *Lb. rhamnosus* GG. Differences were observed both in terms of the total protein yield as well as the protein patterns. According to the author, among the methods tested LiCl allowed maximum recovery and appeared to be the most suitable extraction method. Concerning the cases where molecular methods are used to study the role of S-layer proteins,

it has been reported that deletion of the genes encoding the SlpA S-layer protein in *Lb. acidophilus* NCFM was linked to a concomitant decrease in the adhesion of the bacteria cells to Caco-2 cells, could have also resulted from the removal of multiple surface-associated proteins (Buck *et al*., 2005).

In addition to the S-layer proteins, non-S-layer surface proteins and non-proteinaceous compounds have been associated with the adhesion of lactobacilli to epithelial cells, intestinal mucus, or extracellular matrix components (Deepika and Charalampopoulos, 2010; Jensen *et al*., 2014). Most of early studies involved treating the cells with variety of enzymes, chemicals, and physical treatment and then testing their adhesion ability. Depending on the strain that was studied the main adhesive compounds were probably either proteins or carbohydrates, or a combination of the two, and were either surface-associated or extracellular (Adlerberth *et al*., 1996; Tuomola *et al*., 2000; Lorca *et al*., 2002; Yadav *et al*., 2013; Jensen *et al*., 2014).

Studies have used genome sequences to predict the presence of adhesive components, which they have then purified and cloned (Azcarate-Peril *et al*., 2008). Using the information from the genome sequence it has been shown that mucus-binding proteins from LAB contain several repetitions of a 100-200 amino acid sequence, which has been described as mucin binding domains (Mub), that have only been found in LAB, including pediococci, lactococci, and lactobacilli (Boekhorst *et al*., 2006). Rojas *et al*. (2002) illustrated that an adhesion promoting protein with a molecular weight of 29 kDa is engaged in the binding of *Lb. fermentum* 104R to small intestinal mucus from piglets and to partially purified gastric mucin. A similar sized protein of 29 kDa was found to mediate adhesion of *Lb. reuteri* JCM 1081 cells to HT-29 cells (Wang *et al*., 2008), whereas a high molecular mass surface protein

mucus binding protein Mub, 358 kDa from *Lb. reuteri* 1063, was associated in the interaction between bacterial cells and mucus (Roos and Jonsson, 2002). Adhesion surface proteins have also been identified in strains of *Lb. crispatus* (Antikainen *et al*., 2002), *Lb. salivarius* (van Pijkeren *et al*., 2006), and *Lb. plantarum* (Izquierdo *et al*., 2009).

Although studied to a much lesser extent than proteins, other surface-associated components, such as lipoteichoic acid, have been proposed to play a role in the adhesion of lactobacilli to the intestinal mucosa (Chauviere *et al*., 1992; Greene and Klaenhammer, 1994). In particular, lipoteichoic acid has been shown to act as an adhesion factor for *Lb. johnsonii* to Caco-2 cells (Granato *et al*., 1999) and *Lb. reuteri* (Walter *et al*., 2007).

### 1.2.3 The Genus *Lactobacillus*

Lactobacilli are nutritionally fastidious, and are associated with a large variety of plants and animals, a factor that has presumably contributed to their diversity, by adaptive radiation. Lactobacilli are used extensively for fermentation of plant material, dairy products and meat (Giraffa *et al*., 2010) In addition, some species have potential for production of raw ingredients for industrial processes, such as propanediol production for the textile industry (Nakamura and Whited, 2003). Lactobacilli are part of the normal human GIT microbiota and they may also be found in the GIT tracts of other mammalian species (McCartney and Gibson, 2013; Etzold *et al*., 2014; Rajilić-Stojanović and Vos, 2014). This has added further incentive to detailed microbiological, biochemical and genomic studies of lactobacilli (Ravcheev *et al*., 2013). Taxonomic analysis has already led to a recognition of the unusual diversity of the genus *Lactobacillus* (Canchaya *et al*., 2006).

The first essential step in many food fermentation is the catabolism of carbohydrates by the lactic acid bacteria. *Lactobacillus* as one of lactic acid bacteria group exhibit as enormous capacity to degrade different carbohydrate and related compounds.

There has been considerable research undertaken on lactobacilli as probiotics both in context of gut health and control of human infections, including vaginosis (Antonio *et al*., 2005; Hemmerling *et al*., 2005; Hemmerling *et al*., 2010). However, the focus of the research in this thesis is on *Lb. casei* and the literature review is limited to this and related species that occur in food systems and may impact on gut health.

### 1.2.4 *Lactobacillus* Species in Dairy and Fermented Food Products

*Lactobacillus* species exhibit a great diversity in their metabolism properties as a part of a number of fermented food products. Lactobacilli are added as deliberate starter or take part in the fermentation as natural contamination of the starting substrates. Various species of lactobacilli are used in industry as starter in dairy products and fermented food (Wang *et al*., 2014), fermented milk (Zagato *et al*., 2014), fermented vegetable and fruit juices (Filannino *et al*., 2014) fermented meat (Rubio *et al*., 2014) and in Cheese (Xu and Kong, 2013).

Two main groups of LAB are potentially important and associated cheese-ripening events. The first group, which has been the most studied, include mesophilic (*Lactococcus lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis*) and thermophilic (*Streptococcus thermophilus* and *Lb. helveticus*) starters. For many cheese types (*e.g*. Cheddar, Colby), these starters are the only microorganisms deliberately added to the cheese milk and usually the viable starter densities in cheese one day after manufacture (~$10^8$ - ~$10^9$ cfu/ g cheese) are at least 4-5 log cycles higher than other lactic acid bacteria present in the cheese. Therefore, starters not only contribute significantly to acid production but will also make the only

significant contribution to the biomass of LAB in the young curd. This relatively high starter biomass represents considerable biocatalytic potential for cheese ripening reactions, a potential that could be modulated through autolysis of the starter cells. The second groups of LAB, the adventitious non-starter lactic acid bacteria (NSLAB), are present at low cell densities (~$10^2$ cfu/g, cheese) in good quality curd at one day. However, during ripping the NSLAB proliferate and reach and maintain densities of (~$10^7$ CFU/g ripening, the NSLAB proliferate and reach and cheese for extended periods of time (McSweeney *et al*., 1994).

The NSLAB normally consist of various species of mesophilic lactobacilli and pediococci. The lactobacilli include both homofermentative species (*Lb. casei* and *Lb. plantarum*) and heterofermentative species (*Lb. fermentii* and *Lb. brevis*) (Martley and Crow, 1993).

Lactobacilli showed a potential for use as alternative preservation in specific food application. The property of lactobacilli which has become more appreciated is the ability to produce bacteriocins, which provides the producing organism with a selective advantage in a complex microbial niche. Incorporation of *Lactobacillus* as starters or the inclusion of a purified or semi-purified bacteriocin as an ingredient in food products for preventing pathogen bacteria has been proposed (Coman *et al*., 2014).

### 1.2.5 Molecular Characterization of *Lactobacillus* species

The genus is quite diverse and consists of a number of different species with little commonality and shows large phenotypic, biochemical and physiological variability that can be used as a basis for their differentiation. During the last few years, nomenclature of the *Lactobacillus* genus has evolved and contains to date more than 171 species (Coeuret *et al*.,

2003). *Lactobacillus* common taxonomical features are restricted to their rod shape and ability to produce lactic acid either as an exclusive or at least a major end product. Bacteria differentiation and classification was based on classical phenotypic tests using physiological characteristic such as morphology under light microscope, respiratory type, motility, growth temperature, biochemical characteristics such as homo- or hetero-fermentation and carbohydrate fermentation.

The typing of lactobacilli has generally been conducted by cell and colony morphology and biochemical tests. These techniques type bacteria based on their ability to ferment sugars and produce acids such as lactic acid and acetic acid (Gasser, 1970). Many studies emphasize that the classification of lactobacilli is unsatisfactory and does not reflect the real phylogenetic relatedness of different strains and species (Collins *et al*., 1991; Millsap *et al*., 1997). Several new genetic and chemotaxonomic approaches have been used during the last 14 years with an aim of improving the classification and identification of lactobacilli: for example, analysis of plasmid content (Xi *et al*., 2013), sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of whole-cell protein and sequencing of rRNA (Grosu-Tudor *et al*., 2013). All of these approaches have improved the taxonomic knowledge of the generic and suprageneric relationships of lactobacilli.

### 1.3 *Lactobacillus* and Health Aspects: Rationale and Mechanisms of Action

Several lactobacilli have been recognized as probiotics due to their wide range of health promoting effects in humans. However, little is known about the molecular mechanisms underpinning their probiotic functions. Some strains are able to degrade carbohydrates such as lactose that may cause abdominal pain. In addition, it was shown that some lactobacilli strains can improve mineral absorption in Caco-2 cells (Gilman and Cashman, 2006).

Lactobacilli can also contribute to improve the nutritional status of the host by producing different group of vitamins (Fukushima *et al*., 2007). More recently, the role of lactobacilli in energy homeostasis, particularly in obese patients, is the object of increased research interest (Turpin *et al*., 2010). Lactobacilli are also involved in the prevention of diseases. The ability of some strains to bind to intestinal cells, their pathogen-associated molecular patterns and the metabolites they produce confer interesting immunomodulatory effects. Finally, pathogenic fungi, virus or bacteria can be inhibited by some of lactobacilli species.

Cell walls are an important structural component of prokaryotic organisms and essential for many aspects of their life. Particularly, the diverse structures of the outermost boundary layers strongly reflect adaptations of organisms to specific ecological and environmental conditions (Beveridge and Graham, 1991). Over the past two decades of research, it has become superficial that one of the most common surface structures on archaea and bacteria are monomolecular crystalline arrays of proteinaceous subunits termed surface layers or S-layers (Sleytr, 1978, Sleytr *et al*., 1993).

### 1.3.1 Cell wall and Surface properties of *Lactobacillus*

The surface properties of *Lactobacillus* are of significant technological importance as they determine the interaction of the bacterial cells with the host, and therefore influence their location in the gut and their functionality. Studying the surface of the bacteria is critical for understanding the adhesion process better. The cell wall of Gram-positive bacteria, including *Lactobacillus*, is a rigid structure outside the plasma membrane that protects them from osmotic lysis and toxic substances. It consists of peptidoglycan, which is abundantly decorated with a variety of compounds, including teichoic and lipoteichoic acids, proteins, and polysaccharides (Schär-Zammaretti and Ubbink, 2003).

The surface-associated proteins can be divided into those that are associated with the membrane/cell wall, either through covalent or non-covalent interactions. The first category includes proteins that are bound to the cytoplasmic membrane by hydrophobic transmembrane domain(s), lipoproteins which are bound to the cytoplasmic membrane, and proteins that possess a C-terminal covalent LPXTG motif and which are bound to the peptidoglycan network by sortases. The second category includes proteins that are attached to the cell surface by non-covalent associations, by recognizing some cell-wall-binding domains (Desvaux *et al*., 2006, Sánchez *et al*., 2008).

The S-layer is a unique class of non-covalently attached proteins that form a structured layer on the surface of many bacteria and Archaea. The bacterial S-layer is a monomolecular crystalline array composed of protein or glycoprotein subunits and has a size between 40 and 200 kDa. In lactobacilli, the S-layer forms a two-dimensional structure over approximately the whole cell surface (Schaer-Zammaretti and Ubbink, 2003). The S-layer proteins in

lactobacilli are usually their molecular weights range between 25 and 71 kDa, depending on the strain (Åvall-Jääskeläinen and Palva, 2005).

The S-layer proteins from *Lb. brevis* and *Lb. buchneri* are reported to bind to the polysaccharide of the cell wall (Masuda and Kawata, 1981), but the location of the proteins is unknown. In contrast, the cell wall binding protein of  domain of *Lb. acidophilus* ATCC 4356 SlpA and *Lb. crispatus* JCM 5810 CbsA has been determined to reside the C-terminal one third of these proteins (Smit *et al*., 2001, Antikainen *et al*., 2002). The literature also shown that not all *Lactobacillus* species has the S-layer proteins and that include *Lb. casei,* while lacking known Slp genes, LiCl extracts of *Lb. casei* strains have been shown to contain proteins that may be surface located (Boot *et al*. 1996; Zhang *et al*. 2009; Hynonen and Palva 2013).

### 1.3.2 Competition between Probiotic and Pathogen on Adhesion Receptors

The epithelial cells of the intestinal lining are covered by a protective layer of mucus, consisting of glycolipids and a complex mixture of large and highly glycosylated proteins called or mucins as the main components (Deplancke and Gaskins, 2001). Mucin expression and composition are dynamic, balanced between production by goblet cells and degradation by (*e.g.* human and/ or bacteria origin) and physical erosion in the gut due to transit functions (Deplancke and Gaskins, 2001). Bacteria adhering to mucus might therefore have a short residence time. In this way, the mucus layer has the potential function of protecting the host against undesirable bacterial colonization (Ouwehand *et al*., 2001; Servin, 2004).

### 1.3.3 Probiotics and the Immune system

The interrelationship between microbiota and host organism can be either symbiotic or commensal (Backhed *et al*., 2005; Chu and Mazmanian, 2013). The microbiota, as mentioned in section 1.2.1, is essential in facilitating the absorption of nutrition and preventing the colonization by pathogenic bacteria entering the human body. Consequently, it is essential that the immune system recognises components of the microbiota as such and establishes a state of tolerance towards them. Specific strains of probiotics can influence many mechanisms in the intestinal *lamina propria* (Palomar *et al*., 2014).

However, research evidence shows that one should not consider members of the GIT microbiota as simply independent residents of the host, but relatively important contributors in defining the gut ecosystem (Kelly and Conway, 2001). Previous research has implicated non-pathogenic bacteria as factors regulating mucosal inflammation (Kelly and Conway, 2001) and host defence (Gilmore and Ferretti, 2003). Enteric bacteria have also been established to supply energy substrates to epithelial cells, such as short-chain fatty acids produced through bacterial fermentation. Indeed, an essential partnership has evolved between gastrointestinal epithelium cells, immune cells, and resident bacteria from which the normal function and activity of each is dependent on the other two components (McCracken and Lorenz, 2001).

## 1.4 *Lactobacillus* and Stress Responses

A research topic of interest has involved the study of the adaptation mechanisms of *Lactobacillus* species to stressful environmental conditions (De Angelis and Gobbetti, 2004a;

Golod *et al*., 2009; Hamon *et al*., 2014). This interest is promoted by the commercial demand for fermented products containing viable *Lactobacillus* and other probiotic cultures. The desire is to maximise viability, allowing for product shelf life as well as efficacy in terms of providing probiotic benefits.

*Lactobacillus* cultures, generally of human origin, are usually grown to high densities in artificial media, then dried and added to a food matrix and stored, before being subsequently ingested and exposed to gastric acid and the human intestinal environment. As such, probiotic strains in this cycle may experience a wide variety of differing stress including heat, cold, oxidation, osmotic, acid or bile and starvation stress. Like all other living organisms, lactobacilli have evolved defence systems to cope with environmental stress. Understanding the detailed molecular mechanisms underlying these systems and how they can be exploited to allow optimization of the production of probiotic cultures that maintain high levels of viability through to the target site in the intestine is of research and commercial importance.

Fermented dairy products, most notably fermented milk drinks and yogurts are the most commonly used food systems for delivery of probiotics to the consumer. Recent years, a number of studies have investigated the potential of probiotic dairy food developments in such diverse food systems as Cheddar cheese (Hamon *et al*., 2014), cereal bars (Ouwehand *et al*., 2001), spray-dried dairy powders (Jantzen *et al*., 2013), table olives (Lavermicocca *et al*., 2005) and fermented sausages (Jantzen *et al*., 2013). In this respect, a convenient means of introduction of probiotic culture during product manufacture is in a dried powder form. In general, it is considered desirable that probiotic for human use should be of human intestinal origin (Dunne *et al*., 2001). As such, these bacteria usually reside in relatively stress-free, nutrient-rich, anaerobic environment of natural pH (Vaughan *et al*., 2005). However, when such intestinal bacteria are selected for probiotic use in food, they are typically exposed to a

verity of stressful conditions. These include temperatures extremes, for example during freeze drying or spry drying, and acid exposure, as occurs during storage in probiotic fermented food products, and while in stomach following ingestion. It is thus important to know not only which conditions are favorable or unfavorable for the life of *Lactobacillus* but what mechanisms permit their survival and metabolic activities under stress conditions. The field of environmental stress responses of *Lactobacillus* spp. has been steadily increasing in recent years.

Understanding stress responses in *Lactobacillus* spp. may permit: (a) tools to be developed for showing tolerant or sensitive strains; (b) their increased use in food processes and for medical applications through the optimization of growth, acidification, proteolysis, bacteriophage resistance, bacteriocin synthesis and probiotic effects, (c) their improved growth and/or survival by suitable methods or by use of genetic engineering to build new food-grade starters; and (d) the fitness and level of adaptation of culture (De Angelis and Gobbetti, 2004a). Environmental stress responses in *Lactobacillus*, which have been investigated mainly by proteomics approaches, are reviewed here.

### 1.4.1 Oxidative Stress

Lactobacilli bacteria can be affected by oxidative stress at a range of stages in their production, including fermentation, drying, and storage (Corcoran *et al*., 2008; Mills *et al*., 2011). Moreover, they may be exposed to steep gradients of oxygen in the GIT, particularly at the mucosal surface (Cummings and Macfarlane, 1991). Oxygen alone is incapable of damaging the cell, however, partial reduction to water results in reactive oxygen species, such as the superoxide anion radical ($O_2$-), the hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide

($H_2O_2$), which can affect probiotic activity and viability. Radicals can react with and damage proteins, lipids and DNA oxidatively (Corcoran *et al*., 2008).

Lactobacilli are fermentative, anaerobic microorganisms which do not use a proton translocation electron transport chain but possess oxidases which utilize $O_2$ to oxidize substrates such as pyruvate (Sedewitz *et al*., 1984) or NADH. The NADH oxidase/NADH peroxidise system is an alternative way to regenerate $NAD^+$, along with the conversion of pyruvate to lactate or ethanol. Pyruvate is then available for conversion to acetate, yielding an extra mole of ATP (Sedewitz *et al*., 1984). *Lb. delbrueckii* subsp. *bulgaricus* can reduce $O_2$ to $H_2O_2$ with a NADH-dependent oxidase to eliminate or lower the level of $O_2$. The same was found for *Lb. plantarum* ATCC 8014 through the oxidation of NADH, *D/L*-lactate or pyruvate. Three moles of NADH were consumed per mole of $O_2$, indicating that $O_2$ was reduced to $H_2O$ via $H_2O_2$, by a NADH-dependant oxidase and a NADH-dependant peroxidase. Pyruvate oxidation with $O_2$ leads to the formation of $H_2O_2$, while lactate oxidation produces $H_2O$ (Sedewitz *et al*., 1984).

### 1.4.2 Osmotic Stress

Bacteria may suffer exposure to conditions of osmotic stress that causes excessive passage of water out of the cell leading to death (Munns *et al*., 1983). In order to retain turgor during osmotic up shift to a level that supports viability, cells needs to adjust their intracellular osmolyte concentrations. The principle cause of osmotic stress in lactobacilli is salt such as NaCl or KCl, since cells are unable to accumulate $K^+$ or $Na^+$ in sufficiently high concentrations to maintain turgor (Glaasker *et al*., 1998). *Lactobacillus* synthesizes extremely low levels of compatible solutes, if at all, so that transport is a necessary part of taking up these solutes to enhance osmotic tolerance (Poolman and Glaasker, 1998).

Transport systems are ATP-binding cassettes (ABC) such as OpuA or BusA, or ion motive-force driven transporters (BetP, ProP). During osmotic stress mechano-sensitive channels are activated (van der Heide and Poolman, 2000). The transporter possibly responsible for glycine betaine accumulation in *Lb. plantarum* is QacT, an ATP-dependent uptake complex, with high affinity for glycine betaine and carnitine and low affinity for proline (Glaasker *et al*., 1998).

Two-dimensional gel electrophoresis analysis of the osmotic response of *Lb. rhamnosus* HN001 showed that increased expression of GroEL (3-fold) and DnaK occurred following osmotic stress (Prasad *et al*., 2003). Transcriptional analysis revealed that exposure of *Lb. helveticus* to NaCl (4% w/v) increased HtrA expression 8-fold, although this concentration of NaCl did not affect the growth rate of *htrA* mutants (Smeds *et al*., 1998). Wu *et al*. (2013) compared the growth and protein expression patterns of *Lb. casei* Zhang with and without bile salts. Analysis of the differentially expressed proteins showed that several pathways are involved with a complex physiological response under bile salts stress, particularly including protein synthesis and folding (EF-Tu, DnaK and GroEL), modifications to the cell envelope and nucleic acid (NagA, GalU, and PyrD), and key components of central metabolism (PFK, PGM, CysK, LuxS, PepC).

### 1.4.3 Heat and Cold Stress

The ability to adapt rapidly to changes in their environment is essential for the survival of all microorganisms. Temperature is a critical environmental factor, and different organisms have different capacities to respond to temperature change. The heat shock response is the adaptive response to a rapid change in temperature. This adaptive response

leads to the transiently improved expression of a subset of genes, the so-called heat shock genes, which encode heat shock proteins (Hsps) (Desmond *et al*., 2004; Rul *et al*., 2013).

Heat stress affects a number of processes crucial to microbial activity and growth. The principle target of heat is the bacterial membrane, where fatty acids are prone to heat damage and subsequent aggregation of proteins and damage to ribosomes and RNA also occurs (Teixeira *et al*., 1997). Generally lactobacilli are sensitive to temperatures above 50 $^{\circ}$C, although the thermal tolerance appears to be strain and species specific (Gardiner *et al*., 2000). Like other prokaryotes, lactobacilli utilise the well-known heat shock chaperones GroES, GroEL and DnaK (Desmond *et al*., 2004; Prasad *et al*., 2003; Liu *et al*., 2014). N-terminal sequencing of over-expressed proteins in heat shocked *Lb. plantarum* cultures (42$^{o}$C for 1 h) showed up-regulation of DnaK, GroEL, histone-like DNA-binding protein HlbA (HU-beta), and cold shock protein CspC (De Angelis *et al*., 2004b).

Considering their optimal growth temperatures, lactobacilli are mesophilic or psychrotolerant. During industrial processes, like frozen storage of starter cultures, low temperature fermentation during cheese ripening and refrigerated storage of fermented products. Lactobacilli play an important role in the food industry because of their extensive application as starter culture in many fermentation processes. Moreover, lactobacilli also exposed to cold stress during low temperature fermentation and storage of fermented products prior consumption. Cold alters (a) the DNA supercoiling and (b) the stability of at least few mRNA which encode proteins involved in the cold shock response (Champomier-Verges *et al*., 2002). It was reported that the cytoplasmic membrane, the nucleic acids and the ribosome are implicated in sensing the temperature in bacteria (Champomier-Verges *et al*., 2002).

It was shown that cold pre-treatment can improve LAB (Champomier-Verges *et al*., 2002) survival to freezing/thaw cycles. Using a proteomic approach, the rapid induction of specific sets of protein upon cold shock was observed in a variety of bacteria. Among lactobacilli, cold shock responses of *Lb. lactis* and *Lb. plantarum* have been studied at the genetic level. Two dimensional gel-based electrophoresis was used to study *Lb. lactis* in this respect and revealed 22 and 24 proteins called cold induced proteins (Wouters *et al*., 2000), which included proteins with roles in protein translation, sugar metabolism, chromosome partitioning and signal transduction.

Beaufils *et al*. (2006) compared the cold shock response of several carbon catabolite repression mutants of *Lb. casei* to that of the wild-type strain. Following a shift from 37 °C to lower temperatures (20, 15 or 10 °C), all mutants showed significantly reduced growth rates. Moreover, glucose-grown mutants unable to form phosphotransferase system proteins Hpr (phosphocarrier) and the associated protein serine kinase HprK exhibited drastically increased sensitivity to freeze/thaw cycles. However, when the same mutants were grown on ribose or maltose, resistance to freezing and thawing was similar to the wild-type strain. These results suggested a direct interaction of HPr, or one of its phospho-derivatives, with CspA and/or another undetected cold shock protein in *Lb. casei*.

### 1.4.4 Acid stress

Acid resistance is accepted as one of the most important properties for selecting potentially probiotic strains of bacteria. This owes to them being confronted with acidic environments, including in fermented dairy products and in the stomach during gastrointestinal transit. This is important for delivery of probiotic strains since a large ingestion of viable bacteria is necessary in order to achieve colonisation of the lower parts of

the intestine and to function probiotically (Cotter and Hill, 2003; Hamon *et al*., 2014). The growth of lactobacilli is distinguished by the generation of acidic end products of fermentation, which accumulate in the extracellular environment. The pronounced organic acid production of these bacteria creates an environment unfavourable for many other organisms (Anyogu *et al*., 2014). This characteristic is the basis of numerous methods of food preservation by fermentation. These bacteria can also encounter an acidic environment in the stomach after consumption and the development of probiotics renewed the interest for lactobacilli survival in the digestive tract. The carcinogenicity of oral lactobacilli is directly related to their acidogenicity (ability to produce acid at low pH) and aciduricity (capacity to function at low pH) (Harper and Loesche, 1984).

A number of mechanisms regulate the homeostasis of interior pH (pH). The proton-translocating ATPase is the most important for fermentative bacteria (Carlsson, 1997). This enzyme was characterized from *Lb. casei* and its activity was found to be optimal at pH values (5.0–5.5) (Bender and Marquis, 1987; Wu *et al*., 2013). In general proton permeability of the plasma membrane also contributes to the regulation of the $pH_i$. Minimal membrane permeability of *Lb. casei* and *Lb. plantarum* was recorded at pH 4.0 (Bender and Marquis, 1987). One of the more important systems is $F_0F_1$-ATPase. $F_0F_1$-ATP is a multi-subunit enzyme, comprising a catalytic portion ($F_1$) incorporating the (β, α, δ, γ and ε) subunits for ATP hydrolysis and as an integral membrane protein ($F_0$), including the (a, b, and c) subunits. The latter subunits function as membranous channel for proton translocation (Sebald *et al*., 1982). The function of $F_0F_1$-ATPase is two-fold in bacteria, firstly as mechanism for synthesising ATP and secondly as a means of proton extrusion, so that $F_0F_1$-ATPase is crucial for maintaining pH homeostasis at low pH in *Lactobacillus* (Bang *et al*., 2014). Indeed, $F_0F_1$ATpase of lactobacilli appears to function at lower pH value than other LAB. For

example, it has been demonstrated that the $F_0F_1$-ATPase of *Streptococcus thermophilus* had optimal activity at higher pH values compared with that of *Lb. casei* (Nannen and Hutkins, 1991). In addition to $F_0F_1$-ATPase, cation transport ATPase such as $K^+$ ATPase can contribute to pH homeostasis. This complex exchanged $K^+$ for intercellular $H^+$ and converted the transmembrane pH gradient (Kashket, 1987). The $F_0F_1$-ATPase is a well-known mechanism by which LAB are protected against acidic conditions (Corcoran *et al*., 2005).

### 1.4.5 Starvation stress

Survival of lactobacilli during starvation depends on their ability to utilise other energy sources. Starvation conditions were described as leading to the decreased ability of organisms to synthesize ATP, and accumulate nutrients necessary to maintain viability over time (see Nezhad *et al*., 2013). Response to three types of limiting conditions has largely been studied in bacteria: carbohydrate starvation (Hussain *et al*., 2009); phosphate starvation, which is detrimental for the energy supply and DNA/ RNA synthesis; and nitrogen starvation which results primarily in the limitation to protein synthesis (De Angelis and Gobbetti, 2004b). The adaptive response during the exponential phase of growth differs from that used in the stationary phase (Revilla-Guarinos *et al*., 2014). The adaptive response during growth usually involves the induction of specific groups of genes or regulons to cope with a specific stress condition, while the stress response during the stationary phase is mediated by numerous regulons which cope with many stress conditions. Adaptation during the stationary phase does not require exposure to stress to develop the response.

The maintenance of an active metabolic state is common for LAB survival during stationary phase. Catabolism of amino acids plays an important role in the survival of *Lb. sakei* during the stationary phase (Verges *et al*., 1999). Examination of the protein profile showed that starvation induced the synthesis of 16 proteins in *Lb. acidophilus*. Seven of them

were specifically induced by the stationary phase itself while the synthesis of the others was attributed to the low pH (Lorca *et al*., 2001). In response to starvation and nutritional stress, *Lb. casei* has been shown to rapidly and transiently express a characteristic set of proteins that aid survival and protect the cells from fatal damage (Hussain *et al*., 2009). Most of the proteins were involved in glycolytic pathways, centring around pyruvate metabolism. However, the studies on *Lb. casei* nutritional stress were not comprehensive as these were limited by the technical limitations of the number of proteins separated by 2D-gel electrophoresis.

### 1.5 Thesis Objectives

The aim of this thesis is to provide a comprehensive understanding of the growth and cellular responses of *Lb. casei* subjected to conditions relevant to starvation and growth at low pH, conditions encountered in various fermented foods. Understanding the influence of these factors on the growth, survival and functionality of the *Lb. casei* will assist efforts to maintain viability of *Lb. casei* and extend its utility as a beneficial dietary adjunct and fermentation processing aid. The following objectives are presented as the goals of this thesis:

1. To observe the growth of *Lb. casei* GCRL163 under different levels of lactose in the presence and absence of Tween 80 and evaluate the physiological responses utilising proteomic and metabolomic approaches. *Lb. casei* GCRL is cheese isolate and was studied initially by Hussain *et al.* (2009) in context of discovering what carbon sources can be utilized when the principal carbon source, lactose, is depleted in media given that cheese is well known to lack free carbohydrate after the initial period of cheese manufacture. This prior work used proteomics to analyse physiological responses to nutritional stress and there was some evidence that Tween 80 or other C sources present in MRS were being degraded.

The present work was undertaken to explore the nutritional stress responses and survival using more advanced proteomic approaches in a MRS-based medium.

2. To determine whether Tween 80 could be used as a sole carbon and energy source by Lb. casei GCRL163, given that it was suspected from prior work that Tween 80 is utilized as a carbon source during starvation.
3. To observe the growth of Lb. casei at low pH and evaluate physiological adaptations using proteomics, using strains from different environmental sources.
4. The study was conducted to understand the impact of growth at low pH at different growth phases under conditions simulating the pH of fermented food. Two *Lb. casei* strains were used, GCRL163, a cheese isolate, and MJA12, a fermented milk isolate.
5. To examine whether growth under acidic conditions had any impact on the adhesion of *Lb. casei* strain GCRL163 and a presumptive probiotic strain isolated from Yakult (MJA12) followed up by analysis of cell envelope proteins using the LiCl extraction method. Prior research (Nezhad *et al*., 2012) indicated that surface proteins altered when a strain of *Lb. casei* was grown at low pH but there was not information on strain variation or impact on adhesion properties had been published. Given that there is evidence that surface proteins are involved in adhesion and probiotic properties of lactobacilli, this proposed line of research would determine whether environmental conditions influenced adhesion in particular.

# CHAPTER TWO

## Impact of lactose starvation on the physiology of *Lactobacillus casei* GCRL163 in the presence or absence of Tween 80


**Abstract**

The global proteomic response of the non-starter lactic acid bacteria *Lactobacillus casei* strain GCRL163 under carbohydrate depletion was investigated to understand aspects of its survival following cessation of fermentation. The proteome of *Lb. casei* GCRL163 was analysed quantitatively after growth in modified MRS (with and without Tween 80) with different levels of lactose (0% lactose, starvation; 0.2% lactose, growth limiting; 1% lactose, non-growth limited control) using gel free proteomics. Results revealed carbohydrate starvation lead to suppression of lactose and galactose catabolic pathways, as well as pathways for nucleotide and protein synthesis. Enzymes of the glycolysis/gluconeogenesis pathway, amino acid synthesis, pyruvate and citrate metabolism become more abundant as well as other carbohydrate catabolic pathways, suggesting increased optimisation of intermediary metabolism and scavenging. Tween 80 did not affect growth yield, however proteins related to fatty acid biosynthesis were repressed in the presence of Tween 80. The data suggests that *Lb. casei* adeptly switches to a scavenging mode, using both citrate and Tween 80, and efficiently adjusts energetic requirements when carbohydrate starved and thus can sustain survival for weeks to months. Explaining the adaptation of *Lb. casei* during lactose starvation will assist efforts to maintain viability of *Lb. casei* and extend its utility as a beneficial dietary adjunct and fermentation processing aid.



**Keywords:** *Lactobacillus casei*, Proteomics, Starvation, Tween 80, Lactic acid bacteria.

## Introduction

Foods that are commonly used to deliver probiotic microorganisms to consumers include fermented dairy products (particularly fermented milk), yoghurts, and cheese. A number of studies have investigated the potential of Cheddar cheese as a probiotic delivery system because this product is a naturally rich source of probiotic bacterial genera and species (Gardiner *et al*., 1999; Cai *et al*., 2009; Minervini *et al*., 2012). Cheese-associated bacteria have been divided into two broad groups: Starter Lactic Acid Bacteria (SLAB) and secondary microorganisms (Jardin *et al*., 2012). SLAB are involved in acid production during manufacture and contribute to the curding and early ripening processes (Jardin *et al*., 2012). Secondary microorganisms do not contribute substantially to acid production; instead they contribute to cheese ripening and flavour production (Olson *et al*., 1990). Lactic acid bacteria are highly represented in this group, and are commonly referred to as Non-Starter Lactic Acid Bacteria (NSLAB). *Lactobacillus casei* is a dominant NSLAB in cheese, growing internally in most cheese varieties (Beresford 2001 *et al*., Steele *et al*., 2006; Hou *et al*., 2012).

The genus *Lactobacillus* comprises Gram-positive, non-spore forming aero-tolerant anaerobic bacteria (Claesson *et al*., 2007). *Lactobacillus* spp. are generally regarded as safe due to their long association with fermented foods and their natural presence in the small and large intestine (Tuohy *et al*., 2003) and through transient adaptation to the mammalian host can provide health benefits. These probiotic-related interactions are strain specific (Sanders *et al*., 2010) and consequently research investigating strain specific probiotic factors, as well as the use of *Lactobacillus* spp. in food products, particularly cheese, has increased.

Environmental stresses such as osmotic pressure, cold, heat, oxidative, pH and starvation can limit the growth of NSLAB during cheese ripening and in other manufacturing

processes. Among these stresses, nutrient starvation, in particular of fermentable substrates is reportedly the most important limitation to growth (Tsakalidou *et al*., 2011).

It is reported that cheese is deficient in lactose (Ur *et al*., 2004). Lactobacilli, when faced with carbohydrate limitation, modulate several genetic regulons that are associated with different stress responses (Hussain *et al*., 2009). Bacteria adapt to nutrition limitation by assuming a physiological state that is characterized by the down-regulation of nucleic acid and protein synthesis and simultaneous up-regulation of protein degradation and amino acid synthesis (Chatterji *et al*., 2001; Hussain *et al*., 2009).

The physiological effect of lactose starvation and subsequent adaptations on the NSLAB was originally conducted by Hussain *et al*., (2009) and colleagues using a proteomic approach on *Lb. casei* GCRL163, originally is Cheddar Cheese isolate which reported by Chandry *et al*. (1998). Their data were based on 2-dimensional gel-based analyses, which have limits in its capacity to adequately quantify protein abundance and comprehensively examine a proteome (Chatterji *et al*., 2001). Examination of metabolites produced during and after growth in a semi-defined medium suggested lipid degradation occurred as a consequence of starvation, which may have arisen from utilization of endogenous lipids through scavenging or from degradation of Tween 80. Tween 80 is usually added to media to optimize the growth of lactobacilli, however it is not required for the growth of *Lb. casei* (Oh *et al*., 1995). In the present study, the effect of different levels of lactose on the growth and physiological adaptations of *Lb. casei* strain GCRL163 to different levels of carbohydrate starvation was examined using gel-based and liquid chromatography/mass spectrometric-based proteomic approaches. The study also focuses on whether *Lb. casei* GCRL163 has the ability to utilize Tween 80 as a carbon source during starvation by breaking down the

polyethoxylated sorbitan section of the molecule. The goal of the research was to understand more thoroughly the responses of a typical NSLAB cheese strain to carbohydrate starvation as part of the process to develop more effective ways to deliver food-associated probiotic microorganisms and to evaluate the metabolic capacity of NSLAB during starvation phases of fermented food products.

## Material and Methods

**Bacterial strain and growth conditions.** *Lb. casei* strain GCRL163, a Cheddar cheese isolate (Chandry *et al.*, 1998) was obtained from the Gilbert Chandler Laboratory (The University of Melbourne) strain collection and restored at the University of Tasmania under its original name and strain number. The strain was maintained in 40% glycerol in de Man Rogosa and Sharpe (MRS; Oxoid, Australia) broth at -80 °C. Appropriate dilutions of culture grown for 12 h in MRS broth were plated on MRS agar and incubated anaerobically using the Anaerocult A system (Oxoid, Australia) at 37 °C for 48 h. A single colony from the plate was resuspended in MRS broth. One individual colony represented one biological replicate. Cells were passaged through two sequential subcultures in MRS broth incubated at 37 °C for 12 h under anaerobic conditions. Bacterial cells were harvested during late stationary growth phase and washed twice with potassium phosphate buffer (PBS, pH 7.0) and sub-cultured in modified MRS (mMRS) consisting of 1% (w/v) bacteriological peptone, 0.5% (w/v) yeast extract, 0.1% (v/v) Tween 80, 0.5% (w/v) sodium acetate, 0.2% (w/v) tri-ammonium citrate, 0.025% (w/v) $MgSO_4.7H_2O$, 0.005% (w/v) $MnSO_4.4H_2O$ in 0.35 M potassium phosphate buffer at pH 6.2 ± 0.2 also containing either 0, 0.2 or 1% (w/v) lactose. All cultures were incubated at 30 °C for 12 h under anaerobic conditions.

**Starvation experiments.** Buffered mMRS with three different lactose concentrations (1%, 0.2% and 0% w/v) were inoculated with *Lb. casei* GCRL163 to give an initial optical density (OD) of 0.021 ± 0.002 at 600 nm. The cultures were then incubated at 30 °C for 30 days under anaerobic conditions as described previously (Hussain *et al*., 2010). Tween 80 was included in the medium since it reportedly enhances survival. To test the impact of Tween 80 on survival in mMRS broth, media either containing or lacking Tween 80 were also used in these experiments (Corcoran *et al*., 2007). Cell growth was monitored by measuring OD, by determining viable counts on MRS agar and also by calculating biomass yield as dry weight.

Cultures were harvested on days 1, 2, 4, 6, 8, 15 and 30 (150 ml each) using centrifugation at 5,000 rpm for 10 min at 4 °C and the cell pellets washed 3 times with Tris-HCl buffer (pH 7.0). The samples were resuspended in Tris-HCl buffer (40 mM, pH 7.0), concentrating to a standard cell density (equivalent to an $OD_{600}$ of 20).To assess optical density culture suspensions were diluted and the optical density at 600 nm recalculated to eliminate the effect of light scattering. The samples were stored at -80 °C until selected for protein extraction. Three biological replicates were tested for each medium, triplicate cultures each set up from a single colony. Then samples were withdrawn at each time point for proteomic analysis.

For sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), analyses were performed on protein extracts for cells harvested after 1, 2, 4, 6 and 8 days incubation, to determine which samples would be used for subsequent analysis based on evaluating when cultures had entered stationary phase and when further visual changes in protein banding patterns thereafter were not evident. Proteomic analysis by nano HPLC and

tandem mass spectrometry (nanoLC-MS/MS) was performed on cells harvested during early stationary growth phase (day 4 for 1% and 0.2% lactose, day 6 for 0% lactose).

**End-product analysis.** The key end-products of *Lb. casei* GCRL163 grown under different levels of lactose in the presence and absence of Tween 80 were quantified. Formic acid and lactic acid levels in culture supernatants were determined using an Acquity H-series UPLC-Xevo triple quadrupole mass spectrometer fitted with a BEH C18 column (2.1 x 100mm x 1.7 micron particles) (Waters Corp., Milford, Mass., USA). The solvent used for formate was 1% (vol/vol) acetic acid for 1 min followed by a linear gradient to 20% acetonitrile at 4 min at a flow rate of 0.35 ml/min. For lactate the initial hold with 1% acetic acid was 30 s with a gradient to 100% acetontrile at 4 min. The column was held at 45 °C and the sample compartment was maintained at 6 °C. The mass spectrometer was operated in negative ion electrospray mode with a needle voltage of 2.3 kV, and selected ion monitoring was used for formate and selected reaction monitoring for lactate. The ion source temperature was 130 °C, the desolvation gas was nitrogen at 950 L/h, the cone gas flow was 50 L/h and the desolvation temperature was 450 °C. The ion at *m/z* 45.0 was monitored for formic acid with a cone voltage of 30 V and a dwell time of 120 ms. A simultaneous negative ion full scan was acquired from *m/z* 40 to 300 in 200 ms. For lactate the transition from *m/z* 87 to 43 was monitored with a cone voltage of 22V, a collision energy of 7V and dwell of 50 ms. For external standardisation calibration, a 200 µl aliquot of 10 different samples was pooled to create a solution for preparation of the matrix matched spiked standard solutions.

Acetic acid, ethanol, caproic acid and octanoic acid levels in culture supernatants were determined using a Varian HP-5ms column (30m × 0.25mm × 0.25micron) on a Varian 3800 GC coupled to a Bruker-300 triple quadrupole mass spectrometer or a Varian 450-GC

possessing a flame ionization detector. The flow rate used routinely was 1.2 ml/min, the injector temperature was 220°C and 1 μL injections split 10:1 were made. For acetic acid the oven program was 40 °C, held for 2 min followed by a ramp up to 160°C at 25 °C/min. Ions from m/z 35 to 200 were scanned every 0.18 s, and SIM ions at *m/z* 31, 45 and 60 were monitored for 40 ms each. Quality control samples (n=6) at the 0.5% level were run after every 10 samples, and gave an average of 0.47% with a standard deviation of 0.0098% (2.1% RSD). For ethanol, caproic acid and octanoic acid, the injector temperature was raised to 240 °C, and oven program was 45°C for 5 min followed by a ramp up to 160°C at 25 °C per minute and one μL injections split 30:1 were made.

**SDS-PAGE.** Cytosolic proteins were extracted by bead beating 200 μl of the cell suspensions using 0.5 g of zircon beads (0.1 mm; Daintree Scintific, TAS, Australia) in a bead beater for 6 min. Cellular debris was removed by centrifugation at 14,000 rpm for 30 min at 4 °C. The protein concentration in extracts was determined using the BCA kit (Thermofisher Scientific, USA), using bovine serum albumin as a standard. Equal amounts of the sample buffer (1:1) were added to the protein samples and mixed by vortexing for 30 s after which they were heated in heat block at 100 ºC for 10 min. The samples were then centrifuged to remove any remaining cell debris. Six μg of protein was loaded in each lane of the SDS-polyacrylamide gels (resolving gel, 10% and stacking gel, 4%) and run at 100 volts for 90 min. Following electrophoresis the gels were visualized by silver staining (Wilson *et al*., 2012).

**nanoLC-LTQ-Orbitrap tandem mass spectrometry.** Protein samples were analyzed by nanoLC-MS/MS using an LTQ-Orbitrap XL (ThermoFisher Scientific). Equal quantities of each sample (50 μg) were sequentially reduced, alkylated and trypsin-digested

as previously described (Kocharunchitt *et al*., 2012). Normalization of total spectral counts for each biological sample was used to compensate for minor differences in protein concentration, variation in trypsin digestion efficiency between samples and peptide loading. Aliquots of tryptic peptides equivalent to 50% of the in-solution digests were loaded at 0.05ml/min onto a C18 capillary trapping column (Peptide CapTrap, Michrom BioResources) controlled by an Alliance 2690 Separations Module (Waters). Peptides were then separated on an analytical nanoHPLCcolumn packed with 5 micron C18 media (PicoFrit Column, 15 m i.d. pulled tip, 10 cm, New Objective) using a 4-step gradient of 100% buffer A (5% acetonitrile in 0.2% formic acid) to 100% buffer B (90% acetonitrile in 0.2% formic acid) using the following steps: 0-10% B over 7.5 min; 10-25% B over 50 min; 25-55% B over 20 min; 55-100% B over 5 min; holding at 100% B for 15 min and re-equilibration in 100% A for 15 min.

The LTQ-Orbitrap XL was controlled using Xcalibur 2.0 software (ThermoFisher Scientific) and operated in data-dependent acquisition mode where survey scans were acquired in the Orbitrap using a resolving power of 60,000 (at 400 m/z). MS/MS spectra were concurrently acquired in the LTQ mass analyzer on the eight most intense ions from the FT survey scan. Charge state filtering, where unassigned and singly-charged precursor ions were not selected for fragmentation, and dynamic exclusion (repeat count 1, repeat duration 30 s, exclusion list size 500) were used. Fragmentation conditions in the LTQ were: 35% normalized collision energy, activation q of 0.25, 30 ms activation time and minimum ion selection intensity of 500 counts.

**Database searching and criteria for protein identification.** Centroid mode spectra acquired were converted from .RAW files into .mzXML peak list files using the msConvert command (Proteowizard). The extracted MS/MS data were searched against the *Lb. casei* database of 25,421 protein entries downloaded from the National Center for Biotechnology Information on 19/7/2011. Semi-tryptic searches using parent ion and fragment ion mass tolerances of 10 ppm and 0.5 Da, respectively, were performed using X!Tandem running in the Computational Proteomics Analysis System (CPAS), an open-source bioinformatics resource for analyzing large proteomics datasets (Rauch *et al.*, 2006). S-carboxamidomethylation of cysteine residues was specified as a fixed modification and oxidation of methionine was specified as a variable modification. The Peptide Prophet and Protein Prophet algorithms were applied to the X!Tandem search results to assign probabilities to peptide and protein matches, respectively. Peptide-spectrum matches were accepted if the peptide was assigned a probability greater than 0.95 as specified by the Peptide Prophet algorithm. Based on the fit of the data to the predicted distributions of correct and incorrect matches, only peptide-spectrum matches for charge states +2 and +3 were accepted. Protein identifications were accepted if the protein contained two or more unique peptide sequences and the protein was assigned a probability > 0.95 by the Protein Prophet algorithm. This threshold will constrain the protein false discovery rate (FDR) to < 1%.

**Statistical analysis of LTQ-Orbitrap mass spectrometry data.** The spectral count (SpC), a sampling statistic output of Protein Prophet, was used to determine relative protein abundance (Saarela *et al.*, 2004). Differences in the expression level of proteins and their significance was then determined for soluble fractions. $Log_2$-fold changes in protein expression under each treatment condition were calculated based on the average SpC,

according to the method described by Old *et al*. (2005). Significant differences (*P*-value of <0.05) in protein SpC were normalised and tested for significant differences using the beta-binomial distribution test implemented in R. The total spectral count (TSpC) normalisation approach was used as described by Gokce *et al*. (2011).

**Results and Discussion**

*Lb. casei* GCRL163 was grown at three different levels of lactose with the pH of mMRS controlled using potassium phosphate buffer. Bacterial cells were harvested at stationary phase (Figure 1). It has been documented previously that the bacteria generate a general stress response upon entering into the stationary growth phase and become stress cross-protected (Van de Guchte *et al*., 2002; Cohen *et al*., 2006). To understand better how NSLABs adapt to conditions where they are carbohydrate starved this study examined growth in media with and without Tween 80. It has been documented that presence of Tween 80 in the media enhances the growth or survival of lactic acid bacteria (Sidhu *et al*., 1998; Howe *et al*., 1975; Li *et al*., 2004; Corcoran *et al*., 2008).

***Lb. casei* GCRLl63 grows slowly but retains viability during lactose starvation.**

The growth of *Lb. casei* GCRL163 in mMRS supplemented with Tween 80 and containing no added lactose reached maximum viable count after 2 days (Log CFU 8.02) and then viability declined gradually over the 30-day incubation period (final count Log CFU 7.04), while OD remained constant. This indicated that cells remained intact but recovery of culturable cells declined. Dry weight data was consistent with this (Appendix A Figure1). Viability was higher in cultures lacking Tween 80 and lactose. In contrast, cultures containing 0.2% and 1% lactose reached maximum cell density after 2 days (Log CFU 9.18 and Log CFU 9.87 for 0.2% and 1%, respectively), however OD and viability declined

substantially after 6-8 days of incubation (Figure 1 A, B). A similar growth pattern was observed in the absence of Tween 80 (Figure 1 D, E). A decline in pH with 1% and 0.2% lactose growth due to organic acid production (principally lactate, Figure 2) paralleled the changes in OD and cell viability (Figure 1 C, F). Tween 80 did not substantially affect either growth yields or survival whether the media was lactose replete or not.

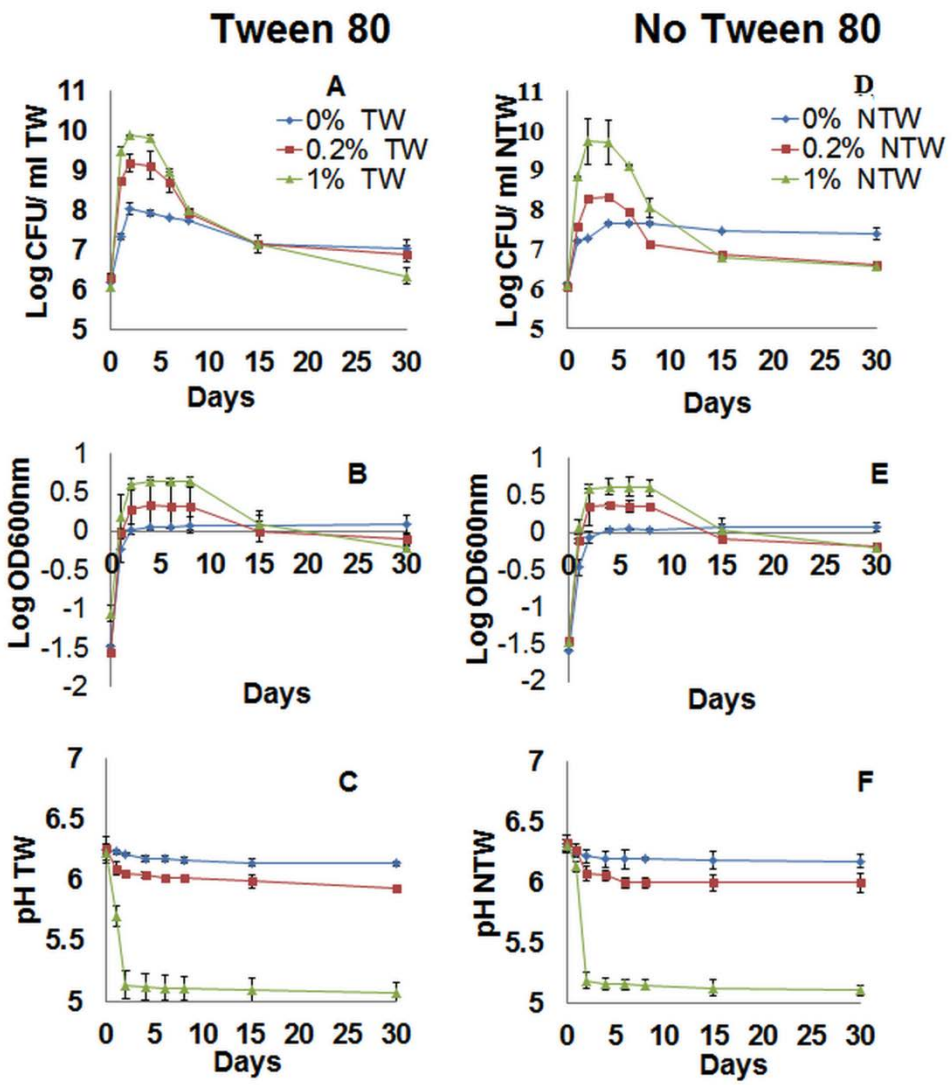


**Figure 1.** Growth of *Lactobacillus casei* GCRL163 in modified buffered MRS broth. Containing 0% (◆), 0.2% (■) or 1% (▲) lactose and either supplemented with Tween 80 (A, B, C) or lacking Tween 80 (D, E, F). Growth levels are shown in the form of viable counts (A, D) and optical density readings at 600 nm (B, E) while pH change is shown in the bottom graphs (C, F).

**Key end-products of starved *Lactobacillus casei* GCRL163.** The key end-products of *Lb. casei* GCRL163 grown on three different levels of lactose in the presence and absence of Tween 80 were measured over an incubation period of 30 days. Lactate and ethanol were the major end products (Figure 2) in media containing 0.2% and 1% (w/v) lactose, reaching maximum levels when stationary growth phase was attained. In the presence of Tween 80 this was found to occur after 6 days, however without Tween 80 the time to reach maximal levels was only 4 days. Lactate and ethanol occurred in a ratio of approximately 10:1, however, when Tween 80 was present ethanol levels were slightly reduced, which was most evident in cultures grown without lactose. The presence of lactate and the reduced pH of the medium may have affected the survival of *Lb. casei* GCRL163 when grown in the presence of high levels of lactose as shown previously by Hussain *et al*., (2010) using a semi-defined medium.

Sustained survival of *Lb. casei* GCRL163 under lactose starvation suggests the use of an alternative carbon source to acquire energy or the cells physiologically adjust to tolerate carbohydrate starvation. The main potential carbon sources in 0% lactose mMRS are citrate, amino acids, acetate and possibly trace carbohydrates in the yeast extract. Formic acid was measured in culture supernatants and data shows that the amount of formic acid produced when cultures lacked lactose, either in the presence or absence of Tween 80, was increased (Figure 2). This is particularly of note, given that the biomass in cultures lacking lactose was significantly lower than for cultures containing 1% lactose.

Hussain *et al*., (2010) showed that long-chain fatty acid compounds, including octanoic and caproic acids, were detected after growth of GCLR163 in a semi-defined medium. The possible source of these long-chain fatty acids is Tween 80, and in this study

the mMRS supplied with Tween 80 compared with mMRS without Tween 80 shows increases in levels of octanoic and caproic acids over the incubation period (Figure 3). This suggests that *Lb. casei* GCRL163 has the ability to degrade Tween 80 weakly but there is no evidence Tween 80 can directly support any significant growth. Although the concentration of these end-products is low, higher levels of both acids may impact on cell viability in cultures containing Tween 80.

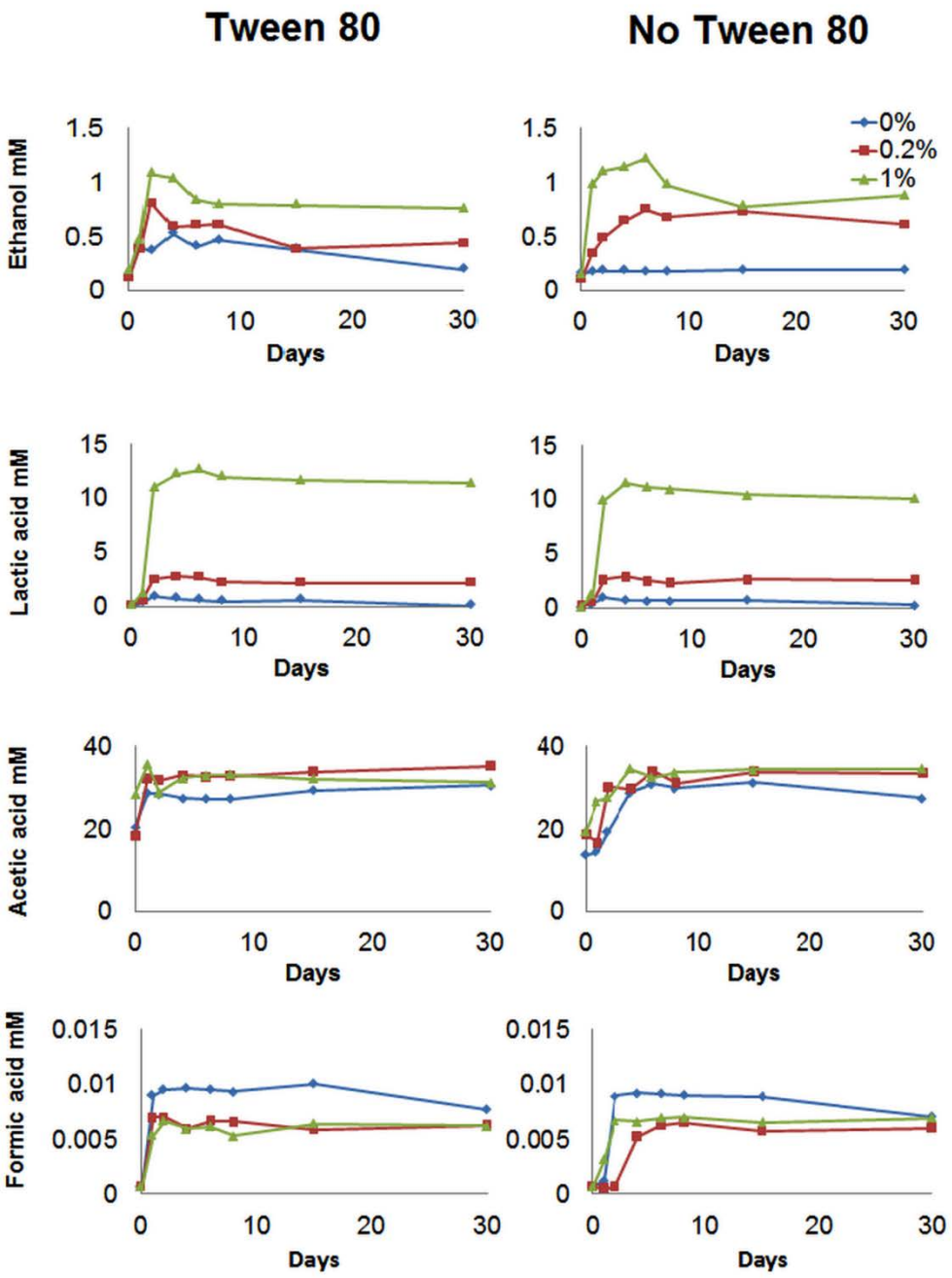


**Figure 2.** Metabolic products formed by *Lactobacillus casei* GCRL163 cultures grown in mMRS with different levels of lactose (0%, 0.2%, and 1% w/v) with and without Tween 80.

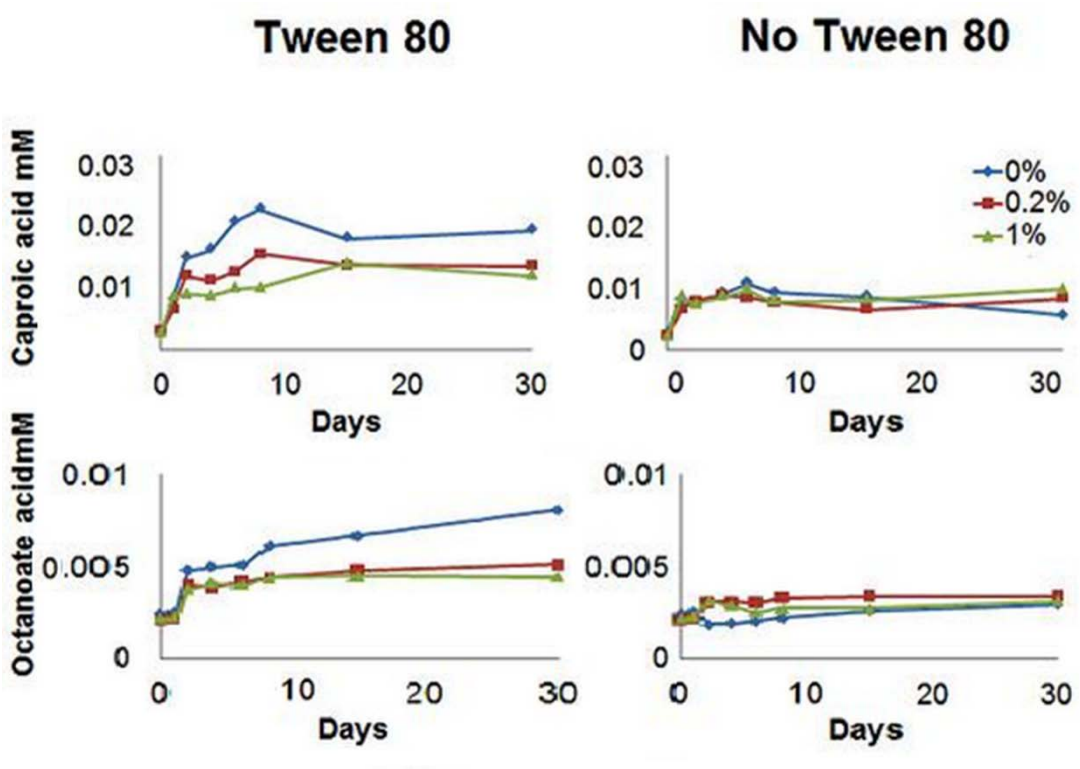


**Figure 3.** Caproic acid and octanoate acid of *Lactobacillus casei* GCRL163 cultures grown in mMRS with different levels of lactose (0%, 0.2%, and 1% w/v) with and without Tween 80.

**The effect of lactose starvation is reflected in broad changes in the proteome of *Lb. casei* GCRL163.** Gel-based protein profiles of *Lb. casei* GCRL163 grown in mMRS with different levels of lactose with and without Tween 80 respectively revealed significantly different expression of a number of proteins (Figures 4 (A) and (B)). This included changes in the intensity of several protein bands, particularly in lactose starved cultures grown with and without Tween 80 in relation to cultures grown with 1% lactose. A consistent set of proteins were differentially increased in abundance in response to starvation that had molecular weight ranges between ~55 and ~80 kDa while several protein bands of 15 to 20, 45 and 120 kDa became less abundant. The SDS-PAGE data demonstrates that lactose starvation resulted in qualitative changes to the overall proteome of *Lb. casei* GCRL163. To determine the nature of these difference within the proteome specifically LC/tandem MS-MS analysis was based on in-solution cytosolic protein samples, allowing discernment of relative abundance changes in detectable proteins within the proteome. Samples from stationary growth phase were chosen for these analyses.

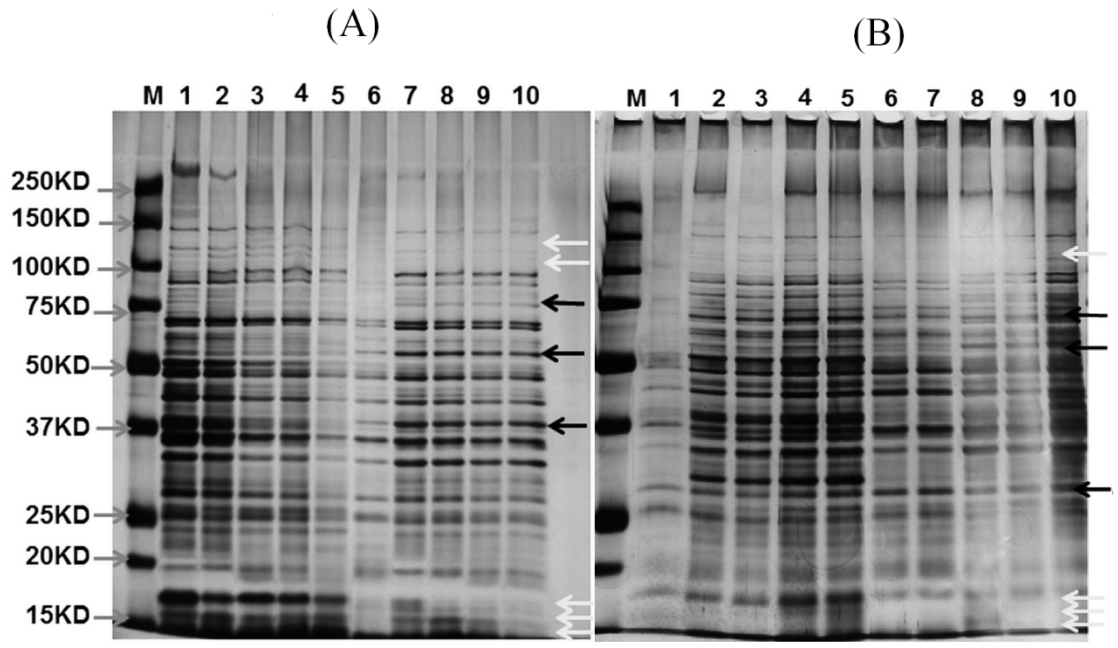


**Figure 4.**

**(A)** SDS-PAGE of cytosolic protein fraction from *Lactobacillus casei* GCRL163 grown in mMRS broth with or without lactose, with Tween 80. The black arrows are proteins bands that are up-regulated. The white arrows are protein bands that are down-regulated.

**(B)** SDS-PAGE of cytosolic protein fraction from *Lactobacillus casei* GCRL163 grown in mMRS broth with or without lactose, without Tween 80. The black arrows are proteins bands that shown qualitative increased concentration. The white arrows are protein bands that show qualitatively reduced concentration.

Lane 1 to 5 are samples from cells cultured in modified MRS with 1% lactose for 1, 2, 4, 6 and 8 days, lanes 6 to 10 are for the cell cultured in modified MRS with 0% lactose harvested at the same days.

**Starvation leads to repressed lactose metabolism and phosphotransferase abundance.** A total of 649 different proteins were detected that passed stringent filtration criteria. The contribution of observed proteins organised on the basis of functional groups is included in Appendix Figure A2. A heat map based on the relative spectral count abundance of proteins grouped into functionally allied protein sets (Figure 5), defined on an ontological basis (de Hoon *et al*., 2004; ; Bowman *et al*., 2012; Kocharunchitt *et al*., 2012) was created to delineate global proteomic changes against cultures that were provided 1% (w/v) lactose. The highest proportions of proteins detected were associated with carbohydrate uptake and subsequent fermentation (18% of proteins identified) and these were separated into distinct sets to better reflect details of the fermentative metabolism. Proteins required for lactose uptake, and catabolism *via* D-galactose, as well as proteins involved in their activation, were strongly repressed in lactose starved cultures (Figure 6).

Francl *et al*. ( 2010) demonstrated that *Lb. gasseri* ATCC 33323 uses PTS systems to import lactose. Lactose-specific PTS proteins, key enzymes required for lactose catabolism (6-phospho-beta-galactosidase, galactose-6-phosphate isomerase LacA and LacB subunits, tagatose 6-phosphate kinase, and tagatose 1,6-diphosphate aldolase, 1-phosphofructokinase), a lactose transport regulator (equivalent to LSEI_0681) and the transcriptional anti-terminator LacT were 5-20-fold less abundant compared to cultures that contained lactose when cultured either with or without Tween 80. When grown with 0.2% lactose *Lb. casei* GCL163 showed virtually no deviation in the abundance (0.7-1.3 ratio to the control) of these proteins. Accompanying the decline in lactose PTS systems, several other PTS protein subunits were also less abundant, likely reflecting the dearth of phosphoenolpyruvate (PEP) occurring during carbohydrate starvation. The spectral count data indicated a 5- to 6-fold increase occurs in the abundance of fructose 1,6-biphosphatase type III, a key regulator of

gluconeogenesis (Titgemeyer and Hillen 2002). Gluconeogenesis would be necessary for creation of key cellular building blocks in the absence of utilisable carbohydrates and is to some extent being maintained or compensated given the lack of PEP that drives the process. The abundance of proteins in the glycolysis pathway and the Entner-Doudoroff pathway were otherwise overall unaffected, suggesting the fundamental importance of these pathways. We had shown previously that 0.2% lactose was growth-limiting but this concentration was clearly sufficient to fully induce expression of lactose utilization genes.

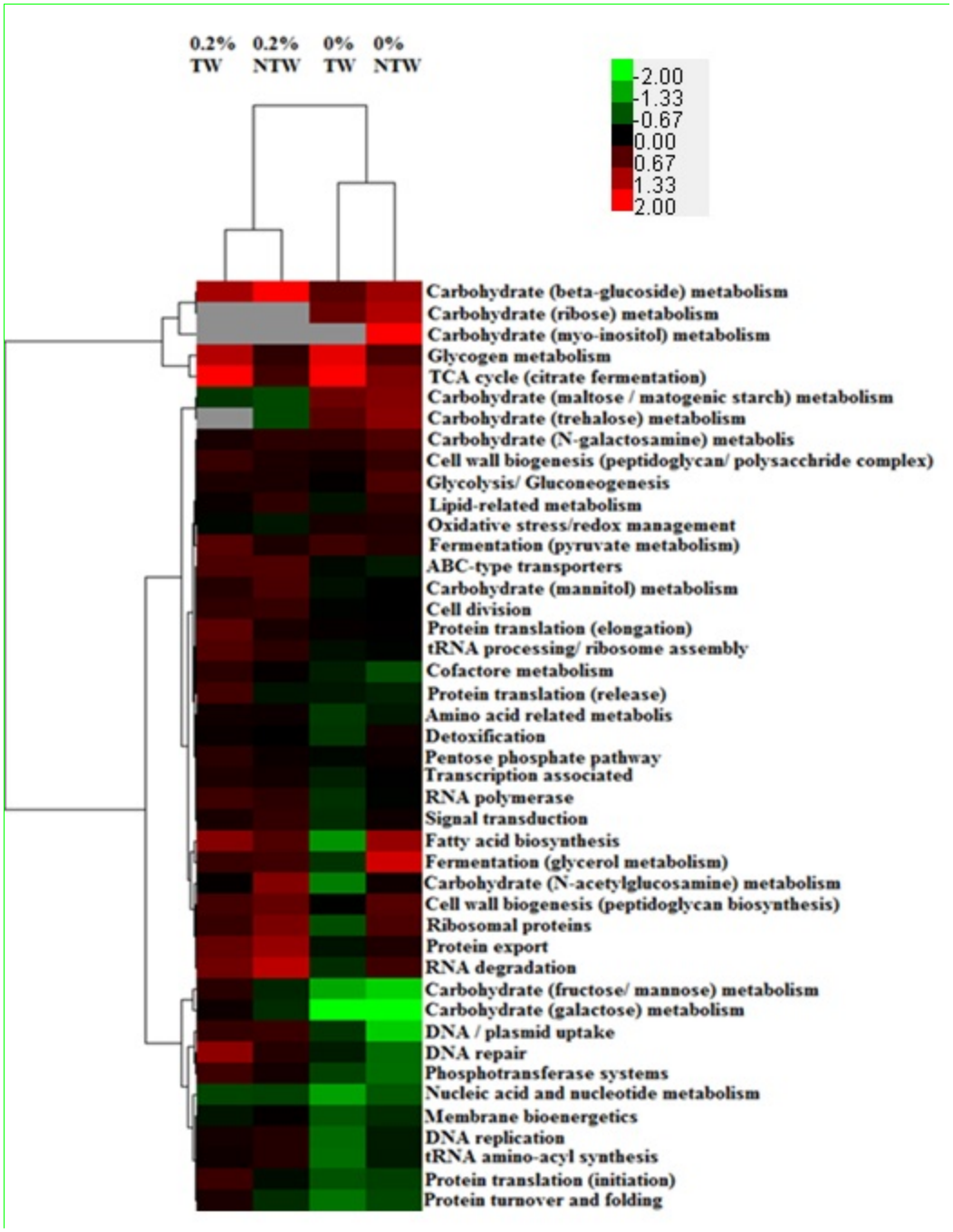


**Figure 5.** Heat map generated from spectral count data organized by functional groups using Cluster v. 3.0.38. Unsupervised hierarchical clustering on the basis of uncentered correlations was used to compare the overall proteome datasets (top dendrogram) and the protein functional groups (left hand dendrogram)a. TW: Tween 80, NTW: No Tween 80.

**Citrate fermentation in the presence of low carbohydrate availability.** Reduced lactose availability and lactose starvation resulted in an overall increased abundance of catabolic enzymes of alternative substrates, however the stimulation was affected by carbohydrate availability as well as the presence of Tween 80. The six proteins (OadAB, CitXFED) that make up the citrate fermentation pathway resulting in the products pyruvate and $CO_2$ had the greatest abundance increases (2-33 fold), when in the presence of Tween 80 (Figure 6, metabolic map). This was unsurprising since the mMRS growth medium contains 8.2 mM triammonium citrate, however the influence of Tween 80 on the protein abundance levels was unsuspected. Utilization of citrate and conversion into end products such as diacetyl and acetaldehyde by *Lactobacillus* species has previously been reported and appears to be strongly subject to catabolite repression (Dudley *et al*., 2005; Diaz-Muniz *et al*., 2006).

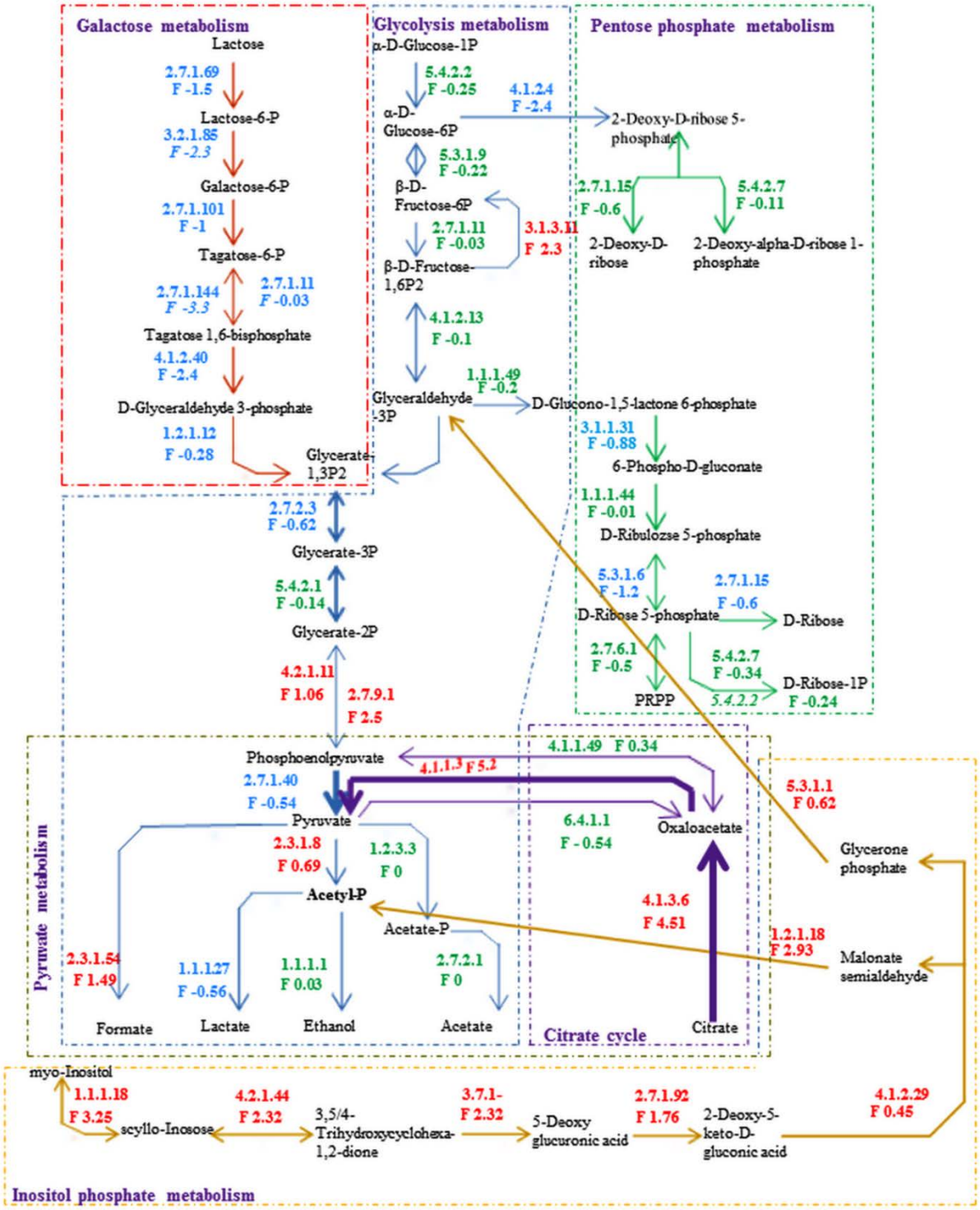


**Figure 6.** Metabolic map of intermediary metabolism of *Lactobacillus casei* GCRL163 under starvation (no lactose, with Tween 80) relative to 1% lactose control in the absence of Tween

80. The map was constructed from spectral abundance data and demonstrates relative abundance of pathway components during starvation and support data related to accumulated metabolic end-products. Pathway assignments of proteins are based on information from the KEGG Database. Enzyme designations are given as their corresponding E.C. code. The width of the line is indicative of the relative abundance of the protein. Red arrows indicate proteins related to galactose metabolism, blue arrows indicate proteins related to the glycolysis pathway, green arrows indicate proteins related to the pentose phosphate pathway, purple arrows indicate proteins related to the citrate cycle, and yellow arrows indicated proteins related to inositol phosphate metabolism. Numerical values underneath the E. C. codes indicate the average log ratio of the fold change in protein abundance with red, green or blue colours denoting significant increases, decreases or insignificant changes in protein abundance.

Metabolism of pyruvate in NSLAB can yield different end-products such as lactate, formate, acetate, ethanol and other compounds that contribute to the particular taste and aroma of cheese. Formation of formic acid in the presence of citric acid when under limitation of other carbon sources such as galactose or lactose has been reported previously (Weinrichter *et al*., 2004; Rehn *et al*., 2011). We observed that the pyruvate formate lyase complex in *Lb. casei* GCRL163 was more abundant under both starved and lactose-limiting growth conditions. The provision of acetyl-CoA to substrate-level phosphorylation *via* acetate kinase (AckA) remained constant regardless of the growth conditions: AckA is an important enzyme which helps to converts pyruvate to different end products and yielding ATP (Kandler *et al*., 1983), so its constitutive synthesis is not unexpected.

**Lactose starvation forces scavenging of alternative carbon sources.** There was also an observably large increase in abundance for most enzymes of the *myo*-inositol catabolic pathway (IolACDEG1G2). This change in abundance only occurred in the absence of Tween 80 (3-17 fold increase), while in other conditions these enzymes were not detectable. An analogous response was also observed for glycerol catabolic enzymes (GlpO, GlpK, GpsA), with Tween 80 also interfering with this response. The effect of Tween 80 potentially relates to its perturbation of lipid metabolism (discussed further below). Glycogen, beta-glucoside, galactose, maltose, trehalose, and ribose catabolic enzymes were also stimulated to lesser degrees under carbohydrate-limited conditions with catabolic enzymes of the latter three substrate more abundant during complete absence of lactose (see Figures 5, heat map, and 6, metabolic map).

In general, lactose starvation increased the abundance of pyruvate-related metabolic enzymes that would suggest a slight promotion of mixed acid fermentation likely related to

citrate fermentation. A 5-6 fold increased abundance of pyruvate orthophosphate dikinase, suggesting gluconeogenesis while at the same time provision of acetyl-CoA, reductant supply and substrate-level phosphorylation occurs *via* acetate kinase (Figure 6, metabolic map). Reduced carbohydrate availability also induced greater abundance of certain peptidase, including dipeptidase A (PepD), Xaa-Pro aminopeptidase (PepQ) and a dipeptidyl aminopeptidase/acylaminoacyl dipeptidase like protein (Appendix A). Beside these proteins amino acid metabolism was largely unchanged or slightly suppressed (AspB, IlveE, MetA, CysK, PepT, PepN) during starvation.

**Tween 80 represses fatty acid biosynthesis-associated enzyme abundance during carbohydrate starvation.** Protein abundance estimated in the present study suggest that in general carbohydrate starvation appears to broadly repress a wide range of cellular functions in GCRL163, including DNA synthesis, replication, repair, and uptake; tRNA charging, protein synthesis and subsequent folding and turnover; and proton-motive-force-driven ATP synthesis. Collectively this repression, though not resulting in a substantially slower growth rate, manifests in lower growth yields when citrate serves as the main source of carbon and energy; this is also coupled to slightly greater cell viability over time (Figure 1).

Based on our proteomic data, the presence of Tween 80 leads to reduced abundance of enzymes involved in fatty acid biosynthesis (Figures 5 and 7) while in the absence of Tween 80 they are instead promoted relative to the 1% lactose grown control cultures. The abundance of other functional protein sets, including cell wall biogenesis, RNA degradation, and ribosomal proteins, are also suppressed to some extent in the presence of Tween 80 during carbon starvation. The reason for the apparent effect of Tween 80 could be due to release of oleic acid *via* non-specific cytosolic esterases. It has been observed that oleic acid

moiety of Tween 80 can be incorporated into the cell membranes of lactic acid bacteria directly (Corcoran *et al*., 2007). This process potentially diminishes the need for the *de novo* synthesis of fatty acids. The mechanistic basis of the effect Tween 80 has on other aspects of cellular physiology is somewhat unclear and requires further investigation. The impact of omitting both citrate and acetate from mMRS, either in the presence or absence of Tween 80, is reported in Chapter 3.

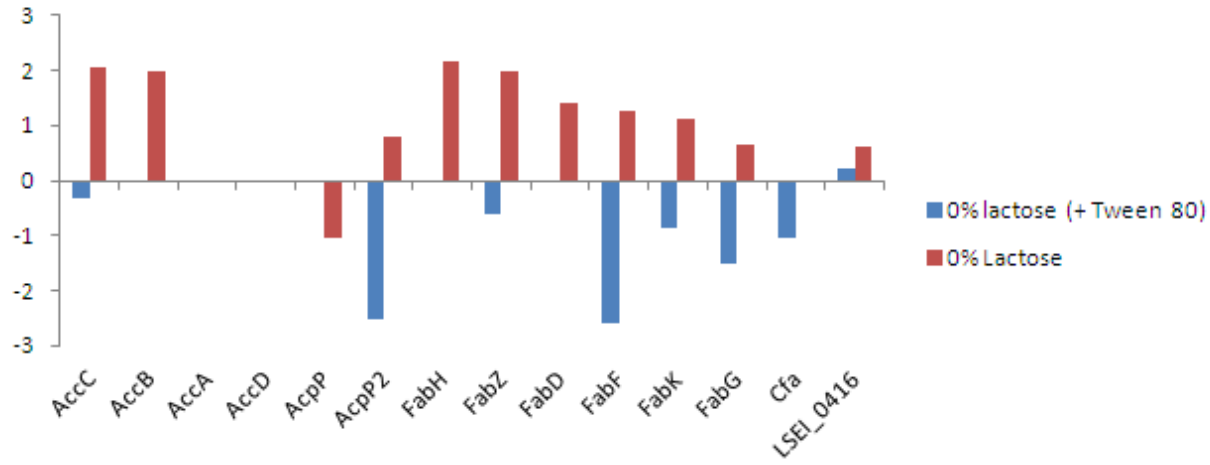


**Figure 7.** Fatty acid proteins with and without Tween 80 in mMRS lacking lactose but containing acetate and citrate.

**Starvation leads to repressed nucleotide synthesis.** Proteins of the purine and pyrimidine biosynthethic pathways were repressed in response to carbohydrate starvation (Appendix A), which likely associates with the resultant slower growth rate. This is likely to be due to reduced leveles of 5-phosphoribosyl-α-1-pyrophosphate availability. The reduced abundance of these pathways is consistent with previous studies in acid and heat stressed Gram-positive bacteria (Koponen *et al*., 2012; Paul *et al*., 2012).

Protein production depends not only on specific regulatory mechanisms, but also changes with growth rate as dictated by environmental conditions.The optimal levels of RNA polymerases and ribosomes necessary or required by cells are tightly regulated (Gyaneshwar *et al*., 2005; Klumpp *et al*., 2009). In the present study, differences in the growth rate of *Lb. casei* GCRL163 under each of the growth conditions were observed. The proteomic analysis revealed a significantly decrease in the production of tRNA amino-acyl synthesases in starved *Lb. casei* GCRL163 (0% lactose with and without Tween 80). The data also indicated that the overall abundance of ribosomal proteins was also significantly reduced under starvation condition (0% lactose, with and without Tween 80). A decrease in cell growth was also reflected in the down-regulation of the cell division proteins FtsX in 0% and 0.2% lactose (with Tween 80) and FtsA in 0% lactose (without Tween 80). Decrease in cell division proteins have been reported by Wang (Wang *et al*., 2012), who showed that acid stressed *Lb. casei* Zhang also showed decreased levels of various cell division proteins.

**Stress-associated proteins are induced during starvation.** Starvation has been shown to induce enhanced physicochemical stress tolerance in *Lactococcus lactis* (Hartke *et al*., 1994). Little equivalent data is available for lactobacilli. Here we can show that starvation leads to increased abundance (2-4 fold) of organic hydroperoxide resistance protein OhrA

and glycine betaine/carnitine/choline ABC-type transporter OpuA. In the absence of Tween 80 there is also a large increase in the protein equivalent to LSEI_0285, annotated as a NADH oxidase in most *Lb. casei* strain genomes. Based on an examination of conserved domains, however, this enzyme appears more similar to CoA-disulfide reductases and thus may fill a thiol-protective role (delCardayre *et al*., 1998) in addition to that provided by glutathione. Overall, the data may suggest starvation results in some level of oxidative stress cross-protection in *Lb. casei* GCL163 as well as possible protection *via* compatible solute uptake above and beyond that provided by the general stress response. In addition to this it was observed that starvation also increased abundance of a peptidoglycan-bound protein homologous (44% identity) to BacA of *Enterococcus faecium*. BacA is a cell wall lysin that is also a bacteriocin activator (Tomita *et al*., 2008). These responses overall potentially favour the survival of *Lb. casei* GCRL163 during carbohydrate limitation.

**Conclusion**

The study provides basic information about *Lb. casei* GCRL163 responses under different carbohydrate-limiting conditions. The data suggests that starvation in *Lb. casei* GCRL163 induces several pathways targeted at cellular energy production. This includes primarily activation of citrate fermentation pathway as well as pathways for the catabolism of polyols including inositol, glycerol, as well as other sugars, likely present in the medium at low levels, or due to triglyceride turnover. It was observed that the presence of Tween 80 affected the abundance of these proteins markedly with citrate fermentation enzymes strongly promoted while carbohydrate scavenging-related proteins only promoted in the absence of Tween 80. Since octanoic acid and caproic acid were detected as end-products, this suggests that this strain has the ability to weakly degrade Tween 80 with resultant metabolites potentially influencing cell physiology, including suppressing fatty acid biosynthesis. Further

research is needed to understand the underlying phenomena resulting in this physiological response. The data shown here contributes to explaining the adaptations of *Lb. casei* GCRL163 undergoes following cessation of primary carbohydrate fermentation.

Because some of the physiological responses did not differentiate between the impact of Tween 80 on lactose starvation, given that the mMRS basal medium contained citrate and acetate as potential carbon sources, further experiments were conducted using mMRS lacking one or both of citrate and acetate in the presence or absence of Tween 80. Results are reported in Chapter 3.

# CHAPTER THREE

# Growth of *Lactobacillus casei* GCRL163 on Tween 80 and impact on low pH tolerance


## Abstract

The non-starter *Lactobacillus casei* strain GCRL163 grew on the emulsifier Tween 80 in the absence of citrate, acetate or a fermentable carbohydrate source in modified carbohydrate-free de Man Rogosa and Sharpe (mMRS) broth, indicating that this strain has the ability to break down and metabolise all or part of the Tween 80 molecule. Growth could not be attributed to abiotic release of oleic acid from Tween 80 during media preparation or culture incubation, as analysis of fatty acids following filtration or autoclaving in stock solutions or mMRS indicated that <0.4% of the oleic acid was released and levels did not increase in uninoculated media during incubation at 30$^{o}$C for 8 days. Gel-free proteomic analysis was undertaken on cell-free lysates for cultures grown on Tween 80 with and without citrate in mMRS broth. Growth on Tween 80 strongly increased the relative abundance of proteins involved in pathways related to glycerol metabolism, the pentose phosphate pathway and fatty acid biosynthesis, while many proteins involved in glycolysis, pyruvate metabolism and protein synthesis were strongly repressed. PTS and ABC transports for ribose/fructose/mannitol were upregulated as were several proteins of unknown functionality, including putative regulators. Proteins involved with riboflavin and pantothenate biosynthesis were also upregulated during growth on Tween 80. The metabolism of Tween 80 appears to have a substantial effect on cell physiology, providing protection against exposure to low pH and, based on the proteomic analysis, does so by strongly promoting fatty acid biosynthesis, protein hydrolysis, amino acid accumulation and stimulation of compatible solute uptake. Furthermore, cell surface proteins were much more

abundant, suggesting modifications to the cell wall and surface. Enhanced abundance of alpha-crystallin Hsp20s, and other proteins related to protein folding (DnaK, ClpE, GroES), following growth in the presence of Tween 80 further suggests that stress cross-protection which aids environmental survival is possibly linked to preventing protein disaggregation.

**Introduction**

Lactobacilli, which are one of the many groups within the lactic acid bacteria (LAB), are generally regarded as safe to humans and play a crucial role in the production of a large variety of fermented foods as well as playing a role in human health (Orrhage and Nord, 1999)

Specific strains of *Lactobacillus* species, including the species *Lb. casei*, which occur in the human mouth and gastrointestinal tract, are currently being marketed as health-promoting cultures, starters or probiotics (Parvez *et al*., 2006; Zhu *et al*., 2009; Kleerebezem *et al*., 2009). *Lb. casei*, along with various other lactobacilli, are naturally aciduric, tolerating low pH and are still able to generate significant levels of organic acids, such as lactate and acetate, at pH levels of <5.0 (Russell *et al*., 1998), which aids food preservation and favors their dominance in acidic foods.

*Lb. casei* strains often occur as non-starter LAB (NSLAB) (Williams *et al*., 2000) and are often opportunistically in food matrices as a result of cross-contamination during the manufacturing procedure or persistence following pasteurization. In the case of cheese production, starter LABs typically consume all the lactose in the cheese matrix in the first two weeks of maturation after which NSLAB increase in the population, growing on other sugars and/or fermenting other substrates including amino acids (Kieronczyk *et al*., 2001), nucleic acids (Thomas, 1986), and citrate (Starrenburg and Hugenholtz, 1991; Diaz-Muniz *et*

*al*., 2006), the end-products of which contribute to the organoleptic properties of cheese and other fermented food products. NSLABs like *Lb. casei* face carbohydrate starvation, elevated acidity, and water activity stress during storage and maturation phases of manufacture. How strains cope with these stressors, and how this impacts on their central metabolism and end-product formation, may influence product traits and subsequent functionality as probiotics. Prior studies on lactose starvation in *Lb. casei* showed that the major impact on the proteome involved proteins in glycolysis or sugar metabolism, where changes were presumably involved in energy yield (Hussain *et al*., 2009). The physiological and metabolic traits of *Lb. casei* strain GCRL163 were studied in a buffered semi-defined medium supplemented with different levels of lactose. Survival of the strain during 30 days in culture without lactose was found to be better than in cultures supplemented with lactose, probably due the latter containing high levels of lactate. Important metabolites detected in starved (0% lactose) cultures were acetate, as a major end product, as well as ethanol, diacetyl, isopentyl alcohol benzyl alcohol, 1-butanol, isopropanol, and 3-(methylthio)-propanoic acid: it was suggested that cells entered a non-culturable state but remained metabolically active and survived under the experimental conditions by utilizing alternative available substrates as energy sources (Hussain *et al*., 2010).

In the previous chapter, it was apparent that *Lb. casei* GCRL163 consumed citrate from the modified MRS (mMRS) growth medium and that end-products formed over time were consistent with Tween 80 being scavenged from this medium. This was demonstrated by testing growth on mMRS in the presence, or absence, of Tween 80, where the results suggested that presence of Tween 80 increased the levels of octanoic and caproic acids produced, suggesting that strain GCRL163 has the ability to weakly degrade Tween 80.

Fatty acid derivatives such as Tween 80, a polythoxylated sorbitan esterified to oleic acid, have been shown to aid in the optimal growth, survival and/or culture stability of LABs (Howe and Ward 1975; Oh *et al*., 1995; Li *et al*., 2004; Corcoran *et al*., 2007). This has been suggested to occur *via* incorporation of exogenous fatty acids leading to enhanced levels of unsaturated fatty acids and cyclopropane fatty acids in the cytoplasmic membrane (Tan *et al*., 2012; Broadbent *et al*., 2014) that appears to provide improved acid tolerance (Corocoran *et al*., 2000; Wu *et al*., 2013).

Previously the physiological effect of lactose starvation and associated proteomic-level adaptations made by *Lb. casei* strain GCRL163 appraised the effects of Tween 80 during conditions of carbohydrate starvation (Al-Naseri *et al*., 2013). The strain studied was chosen since it is a typical Cheddar cheese isolate (Chandry *et al*., 1998) and was identified by multilocus sequence typing as closely allied to the main clonal cluster of *Lb. casei* strains sequence type 1 (Diancourt *et al*., 2007; Ali Al-Naseri *et al*., 2013). It was observed that when GCRL163 was grown anaerobically in modified MRS with 0.1% Tween 80 in the presence of citrate plus acetate, and absence of lactose, fatty acid synthesis proteins were reduced in abundance. Consequently, the current work was undertaken to evaluate whether Tween 80 could be metabolised alone and what impact this had on cell physiology. To further understand the role of Tween 80, analysis of the relative abundance of cell-free extract proteins most affected by Tween 80 in *Lb. casei* strain GCRL 163 was undertaken in buffered mMRS media without acetate but containing Tween 80 and where citrate was supplied as an additional fermentable growth substrate. The data provides an indication of how Tween 80 specifically leads to the manifestation of physiological responses that result in acquisition of a stress tolerant state. Normally the relative abundance of proteins would be compared against a Tween 80 variable in an mMRS base lacking other added carbon sources but little

cell growth occurred in the base medium in the absence of acetate, citrate and Tween 80, so that protein yields would have been too small for proteomic analysis.

**Materials and Methods**

*Lb. casei* GCRL163 originally isolated from Cheddar Cheese. The strain was obtained from the Gilbert Chandler Research Laboratory (The University of Melbourne) strain collection and restored at the University of Tasmania under its original name and strain number. Strains were recovered from glycerol storage by passaging through two sequential subcultures in MRS broth (Oxoid, Australia) incubated at 37 °C for 12 h under anaerobic conditions (Anaerocult A system, Oxoid, Australia). Cultures were plated onto MRS agar and incubated anaerobically at 37 °C for 48 h then starter cultures prepared by suspending a single colony into MRS broth. One individual colony represented one biological replicate.

Bacterial cells were harvested during early stationary growth phase and washed twice with Tris-HCl buffer (40 mM, pH 7.0) and sub-cultured in modified MRS (mMRS) consisting of 1% (w/v) bacteriological peptone, 0.5% (w/v) yeast extract, 0.1% (v/v) Tween 80, 0.5% (w/v) sodium acetate, 0.2% (w/v) tri-ammonium citrate, 0.025% (w/v) $MgSO_4.7H_2O$, 0.005% (w/v) $MnSO_4.4H_2O$ in 0.35 M potassium phosphate buffer at pH 6.5 ± 0.2.

Buffered mMRS at pH 6.5 with no lactose was inoculated with *Lb. casei* GCRL163 to give an initial optical density (OD) of 0.21 at 600 nm. The cultures were then incubated at 30 °C for 8 days under anaerobic conditions as described previously (Al-Naseri *et al*., 2013). To test the impact of Tween 80, citrate and acetate on growth and survival of *Lb. casei* in mMRS broth, media either containing or lacking combinations of Tween 80 (0.1% [v/v]), tri-

ammonium citrate (2% [w/v]), and sodium acetate (0.5% [w/v]) were prepared. Cell growth was monitored by measuring $OD_{600}$ and by determining viable counts on MRS agar.

Cultures were harvested on days 1, 2, 4, 6 and 8 (150 ml each) using centrifugation at 5,000 rpm for 10 min at 4 °C and the cell pellets washed 3 times with Tris-HCl buffer (40 mM, pH 7.0). The samples were resuspended in Tris-HCl buffer (pH 7.0) and stored at -80 °C until selected for protein extraction. Three biological replicates were tested for each medium, with triplicate cultures each set up from a single colony. Proteomic analyses were undertaken for cells grown in media containing Tween 80 (0.1% [v/v either containing or lacking tri-ammonium citrate (2% [w/v]); proteomic analysis was not conducted for the media containing sodium acetate (0.5% [w/v]). Samples from stationary growth phase where used for proteomics analsyses.

**Impact of Tween 80 on *Lb. casei* GCRL163 survival at low pH.** The effect of Tween 80 on the *Lb. casei* GCRL163 survival at pH 2.5 was also tested using buffered mMRS with the presence or absence of Tween 80 (0.1% [v/v]) with the presence of acetate, citrate and 1% lactose. Strains were passed through two sequential subcultures in MRS broth (Oxoid, Australia) incubated at 37 °C for 12 h under anaerobic conditions (Anaerocult A system, Oxoid, Australia). Cultures were plated onto MRS agar and incubated anaerobically at 37 °C for 48 h then starter cultures prepared by suspending a single colony into MRS broth. The mMRS media were inoculated with *Lb. casei* GCRL163 to give an initial OD of 0.21 at 600 nm, the cultures were then incubated at 30 °C for 30 hours under anaerobic conditions as described above. Samples where taken every hour for 30 h and bacterial survival was monitored by determining viable counts on MRS agar.

**Analysis of fatty acids and end-products.** The possible non-biological hydrolysis of Tween 80 in sterile mMRS media and sterile distilled water was quantified by analysis of long chain (C:14 to C:18) fatty acids using the approach outlined by in Quehenberger *et al*. (2011). Free fatty acids were methylated and analysed using the approach described in Nichols and McMeekin (2002), except the instrument used was a Varian 3800 gas-chromatograph coupled to a Bruker 300 triple quadrupole mass spectrometer. Volatile fatty acids (acetic, butanoic, caproic and octanoic) were quantified as previously described using GCMS (Al-Naseri *et al*., 2013).

Tween 80 (VWR, Australia) was prepared at 0.1% (v/v) in distilled water or mMRS broth and either filter sterilized (0.22 μm, Millipore, Sigma, Australia) or autoclaved (121 ºC, 15 min). Triplicate samples were collected daily for eight days (beginning immediately pre-autoclaving or post-filtering) for GCMS analysis.

**Peptide analysis by LTQ-Orbitrap mass spectrometry and protein identification.** Nano LC and MS/MS analysis was performed using an LTQ-Orbitrap XL and Surveyor autosampler and MS Pump Plus controlled by Xcalibur 2.0 software (Thermo Scientific). Peptides were separated and analyzed with minimal void volume LC system using two PEEK Zero Dead Volume (ZDV) tees installed either side of a sample trap column assembly (Upchurch) loaded with a 2.5 cm x 75 μm ID IntegraFrit trapping column (New Objective). Approximately 2 μg of peptide sample was loaded onto the trapping column using 100% mobile phase buffer A (0.1% formic acid) at a flow rate of 20 μl/min over 5 minutes. During sample loading, solvent and unbound analytes were diverted to waste via the downstream ZDV tee. Flow rate was then split down to 200 nl/min using the upstream ZDV tee and peptides were eluted onto a 10.5 cm analytical nanoLC column (New Objective) using a four-

step gradient of 100% buffer B (90% acetonitrile in 0.2% formic acid) to 90% buffer C (90% acetonitrile in 0.2% formic acid). Data-dependent tandem mass spectrometry and data acquisition parameters were essentially as described previously (Al-Naseri *et al*., 2013).

**Results and Discussion**

Our previous study on *Lb. casei* GCRL 163 showed that this strain fermented citrate in buffered mMRS lacking added carbohydrates and that caproate and octanoate accumulated as end-products over the period of growth, indicating that Tween 80 was metabolised (Al-Naseri *et al*., 2013). However, it was not clear that this strain could grow on Tween 80 as a carbon source or whether it was co-metabolised in the presence of other carbon sources in the mMRS, including citrate and/or acetate, plus other carbon sources in tryptone and yeast extract. The ability of strain GCRL163 to grow on Tween 80 in the absence of citrate or acetate was therefore examined (Figure 1). In the absence of Tween 80, citrate and acetate, GCRL163 did not grow significantly (Figure 1D). *Lb. casei* GCRL163 when grown in mMRS containing 0.1% (v/v) Tween 80 as the sole growth substrate reached a population of log CFU/ml of 7.5 after 2 days, with the population slightly increasing for another 4 days reaching a maximum of 7.8 log CFU/ml, then remained constant (Figure 1). The growth yield increased approximately 5-fold when 0.2% (w/v) triammonium citrate and 0.1% (v/v) Tween 80 were present together (log CFU/ml 8.5 after 8 days, Figure 1, panel 2). In the presence of acetate with no Tween 80, *Lb. casei* GCRL 163 showed a 2.63-fold increase in viable counts to log CFU/ml 6.62 after 2 days (Figure 1, panel 4) and numbers did not increase thereafter, indicating that little growth occurred on acetate as a carbon source. However, when acetate was present with Tween 80, viable counts were higher than for Tween 80 alone, indicating co-metabolism.

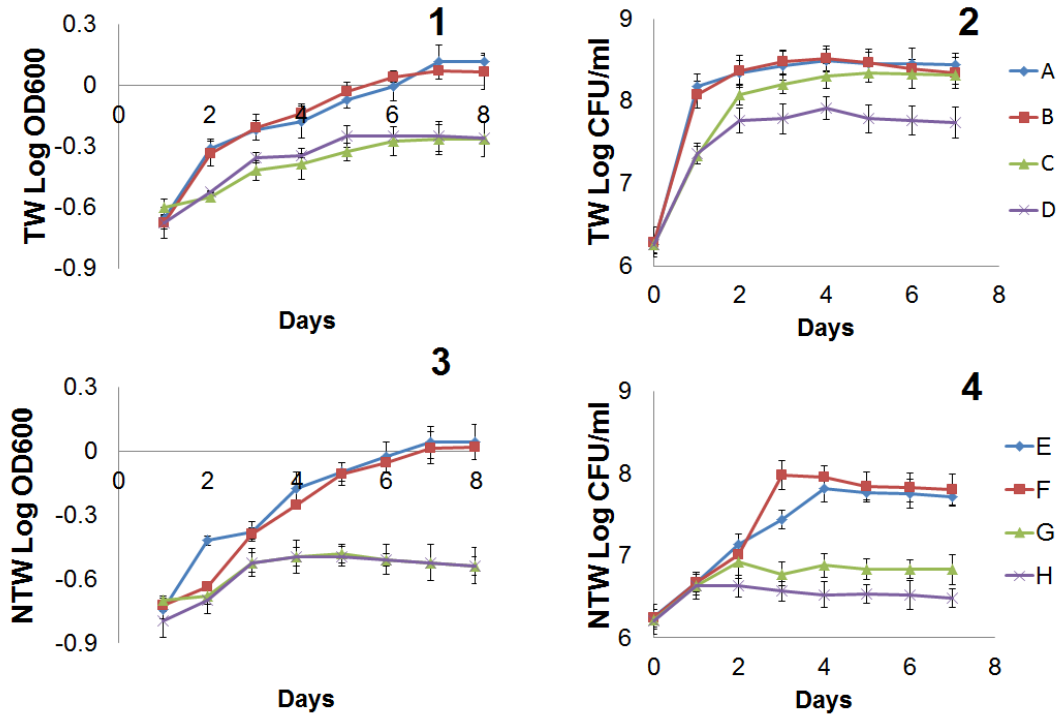


**Figure 1:** Growth of *Lb. casei* GCRL163 in modified buffered MRS broth supplemetation with combinations of Tween 80 (TW), citrate (CIT) or acetate (ACE) and in the absence of Tween 80 (NTW). mMRS either supplemented with Tween 80 (panels 1, 2) or lacked Tween 80 (panels 3, 4). Growth levels are shown in the form of viable counts (panels 2 and 4) and optical density measurement at 600 nm (panels 1 and 2).

***A:*** TW/ CIT/ ACE, ***B:*** TW/ CIT/ No ACE, ***C:*** TW/ No CIT/ ACE, ***D:*** TW/ No CIT/ No ACE, ***E:*** No TW/ CIT/ ACE, ***F:*** No TW/ CIT/ No ACE, ***G:*** No TW/ No CIT/ACE, ***H:*** No TW/No CIT/No ACE, where is TW: Tween 80, NTW: No Tween 80, CIT: Citrate, ACE: Acteate.

In this study proteomic analysis was conducted on *Lb. casei* GCRL163 when grown in mMRS containing 0.1% (v/v) Tween 80 as the sole added carbon source compared to those grown in mMRS containing 0.1% (v/v) Tween 80 and 0.2% (w/v) triammonium citrate. Cell biomass was harvested at day 6 (Figure1) and proteins extracted and analysed *via* nano-LC/tandem MS. A total 1213 proteins with non-singleton peptide identifications were obtained and approximately half of these passed stringent filter criteria described above. When the culture types (Tween 80 alone versus citrate and Tween 80) were compared statistically, 112 and 130 proteins were significantly increased or decreased ($p<0.05$, relative change >2 or <0.5-fold change) in relative abundance, respectively. The following sections describe key changes at a functional level.

**Effect of Tween 80 on catabolic pathways and enzymes potentially involved in its degradation.** Tween 80 is synthesized by dehydration of sorbitol to cyclic ethers, mainly sorbitan and isosorbides, followed by esterification with oleic acid and then condensation with ethylene oxide (Frison-Norrie *et al*., 2001). Tween 80 is susceptible to auto-oxidation (Kerwin 2008) thus it is possible changes in the chemical integrity, especially attack of the olefin component (Kishore *et al*., 2011) enhanced during autoclaving, could lead to easier utilisation by microorganisms (Kerwin *et al*., 2008) or even release products that are utilisable. To determine if the growth observed in Figure 1 was due to breakdown of Tween 80 following autoclaving or during the incubation period of growth at 30 $^{o}$C, GCMS analyses for fatty acids (C:14-18, and unsaturated variants) were undertaken for freshly-prepared Tween 80 in distilled water or mMRS following filtration or autoclaving. mMRS without Tween 80 contained <0.5 mg/l of fatty acids and this did not change throughout the incubation period (8 days) and the average concentration of oleic acid in solutions of Tween 80 in mMRS broth or distilled water before and after autoclaving, and throughout the incubation period, was 3-4 mg/l. This indicated that there was little degradation of Tween 80

from autoclaving and the level of free oleic acid was <0.4% of the Tween 80 provided in mMRS, indicating that growth was due to Tween 80 serving as a carbon source. The production of shorter chain fatty acids as end-products was previously observed over the growth period of 8 days (Al-Naseri *et al*., 2013): with the exception of detecting acetate in mMRS broth supplemented with sodium acetate, volatile fatty acids were undetectable by GCMS and levels did not increase over the incubation period, confirming that the increase in caproic and octanoic acids in cultures was due to biological activity.

Though the nature of the biological degradation pathways involved is so far unknown, a number of enzymes were enhanced in abundance that could be relevant. A number of enzymes were enhanced in abundance that could be relevant to Tween 80 catabolism (Table 1). Based on the proteomic data a preliminary proposed pathway is proposed (Figure 2). Esterases would be needed release the ethoxy acyl and oleic acid side chains from the sorbitan moeity. The activity of the esterase and other enzymes may also be responsible for the production of short chain fatty acids as end-products previously observed in chapter 2 (Al-Naseri *et al*., 2013): this remains experimentally unexplored A predicted esterase of the alpha/beta hydrolase superfamily, equivalent to BN194_18770 (mean normalized SpC of 3.1 ± 2.2 versus non-detection) was detected only in cells grown with Tween 80. Other putative acyl esterases also show enrichment in Tween 80 grown cells (Table 1), however due to the paucity of spectra and variability between biological replicates standard statistical analysis does not confirm whether these observations are reliable at this stage. It was also observed a putative sorbose 1-phosphate reductase (equivalent to BN194_4300) had a 4.3-fold enhanced abundance in Tween 80 grown cells (Table 1) and could potentially be involved in the catabolism of released sorbitan or sorbitan derivative residues since, with side chain removal, a sorbose-like compound would be available. The catabolism would theoretically then

proceed *via* initial phosphotransfer and conversion of the Tween 80 sugar moiety to L-sorbose 1-phosphate by the reductase followed by conversion of sorbitol 6-phosphate to fructose-1,6-biphosphate *via* sorbitol 6-phosphate dehydrogenase (3 homologs present, one increased by 2.7-fold, Table 1). This could represent a source of carbon and energy to the cells from Tween 80. However, a large increase in the abundance of glycerol kinase (GlpK, 44.7-fold increase), glycerol dehydrogenase (GlpD, 10.7 fold increase) was also observed suggesting glycerol may be a major product of Tween 80 hydrolysis. The process by which glycerol is released is unknown but could derive from the ethoxy acyl side chains. Pyruvate oxidase was also observed to become more abundant suggesting pyruvate oxidation *via* acetylphosphate may contribute to ATP generation.

**Table 1.** Enymes putatively involved in degradation and metabolism of Tween 80 in *Lb. casei* GCRL163

| ***Lb. case* loci** | **Gene symbol** | **Protein specific function** | **Fold-increase*** | **P** |
|---|---|---|---|---|
| BN194_30390 | | esterase/lipase, Aes superfamily | 2.8 | 0.116 |
| BN194_11090 | | carboxylesterase, esterase_lipase superfamily | 2.8 | 0.374 |
| BN194_18770 | MqhC | esterase/lipase superfamily protein | 6.3 | 0.117 |
| BN194_07280 | GlpK | glycerol kinase | **44.6** | 2E-05 |
| BN194_07260-70 | GlpD | glycerol-3-phosphate dehydrogenase (aerobic) | **10.7** | 0.0002 |
| BN194_04300 | SorE | L-sorbose 1-phosphate reductase | **4.3** | 0.0088 |
| BN194_28500 | SorD2 | sorbitol-6-phosphate 2-dehydrogenase | 2.7 | 0.113 |
| BN194_11020 | Pgk | phosphoglycerate kinase | **6.7** | 0.0007 |
| BN194_22810 | Pox5_2 | pyruvate oxidase | **8.0** | 0.0 |

*Comparison of protein abundance in cells grown with Tween 80 alone and cells grown with citrate and Tween 80. Fold change values in bold are statistically more abundant ($p<0.01$).

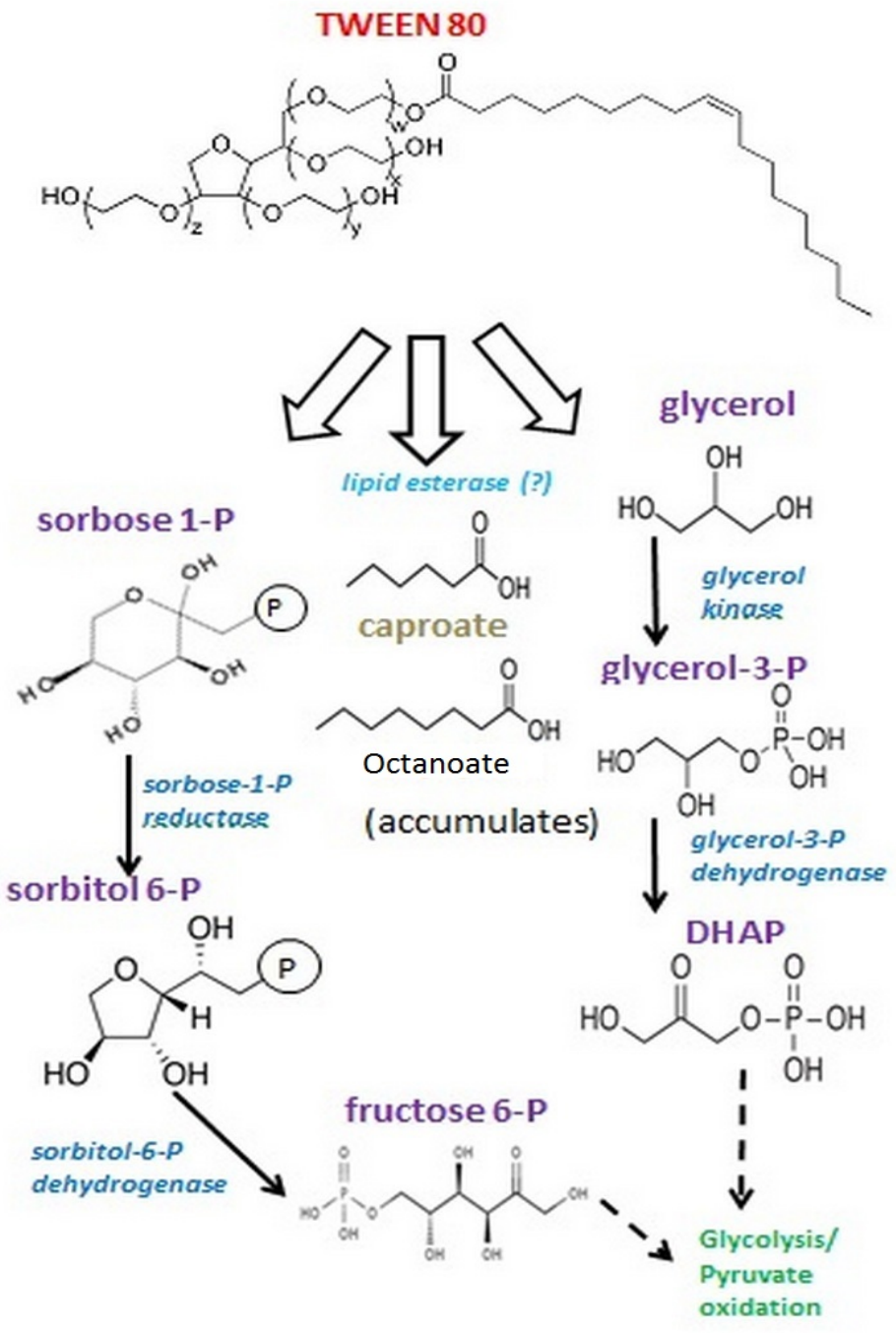


**Figure 2:** Proposed pathway for Tween 80 degradation in *Lb. casei* GCRL163. DHAP = dihydroxyacetone phosphate (glycerone phosphate).

The conversion of L-sorbose to D-sorbitol followed by metabolism *via* fructose-1,6-biphosphate could account for some of the carbon being utilized by cells for growth. Evidence to support this includes the observed increase in relative abundance of proteins involved in the pentose phosphate pathway, including xyulose-5-phosphate-3-epimerase, fructose biphosphate aldolase, 1-phosphofructokinase and xyulose-5-phosphoketolase, for cells cultured in Tween 80 (Appendix B). Hussain *et al*. (2013) observed increases in XpaK and other pentose phosphate pathway enzymes during lactose starvation in *Lb. casei* GRCL163 cells from old stationary phase cultures lacking lactose: this may be attributed to Tween 80 scavening, as the media was MRS modified only to lack lactose or glucose, so once citrate was utilized the relief of catabolite repression may then lead to Tween 80 utilisation for energy production. Several ABC- and PTS-type transporters involved in ribose, fructose, mannose or unkown function were also upregulated in the presence of Tween 80 (Appendix B), which may be associated with transport of sorbitan derivatives. If Tween 80 is not taken up by cells and requires release of oleic acid from the sorbitan core of the molecule, appropriate esterases may well be located at the cell surface or in the extracellular medium and may not have been detected in the cell-free extract analysed in this study. However, a large increase in the abundance of glycerol kinase (GlpK), glycerol dehydrogenase (GlpD) was also observed. A predicted esterase of the alpha/beta hydrolase superfamily, equivalent to LSEI_1694 became substantially abundant, which could potentially release the oleic acid or component molecules from the hydrophilic side-chain (Appendix B).

**General metabolic effects of Tween 80.** Tween 80 though supporting growth due to apparent direct degradation also strongly affects the proteome in relation to that obtained when citrate is also present. Statistical changes in abundance in functionally allied groups of

proteins are shown in (Figure 3) while proteins most altered in abundance are shown in Table 2. In summary slowed growth and less efficient biomass production is indicated by central metabolic pathways being less abundant in tandem with ribosomal proteins and tRNA aminoacyl syntheases. Citrate is clearly much better at supporting growth (Figure 1) and the abundance of citrate lyase and oxaloacetate decarboxylase enzyme complexes required for citrate conversion are consequently highly abundant in citrate grown cells while being nearly absent in Tween 80 grown cells (Table 2). Enzymes associated with end-product formation as part of mixed acid fermentation show some reduction in abundance in Tween 80 grown cells, especially pyruvate formate-lyase (7-fold decrease in Tween 80 grown cells), which suggests less carbon flow *via* pyruvate and subsequent slower growth rates. This is supported by the observation that phosphotransferase system (PTS), involved with phosphorylation of carbohydrates for their subsequent importation and catabolism, are likely much less active since dependent enzyme phosphoenolpyruvate-protein phosphotransferase (PtsI) was less abundant in Tween 80 grown cells (3-fold reduction). Normally this is PEP-dependent enzyme is very abundant during rapid growth on carbohydrates (and also on citrate) where it actively initiates coupled phosphorylation and transport cascades.

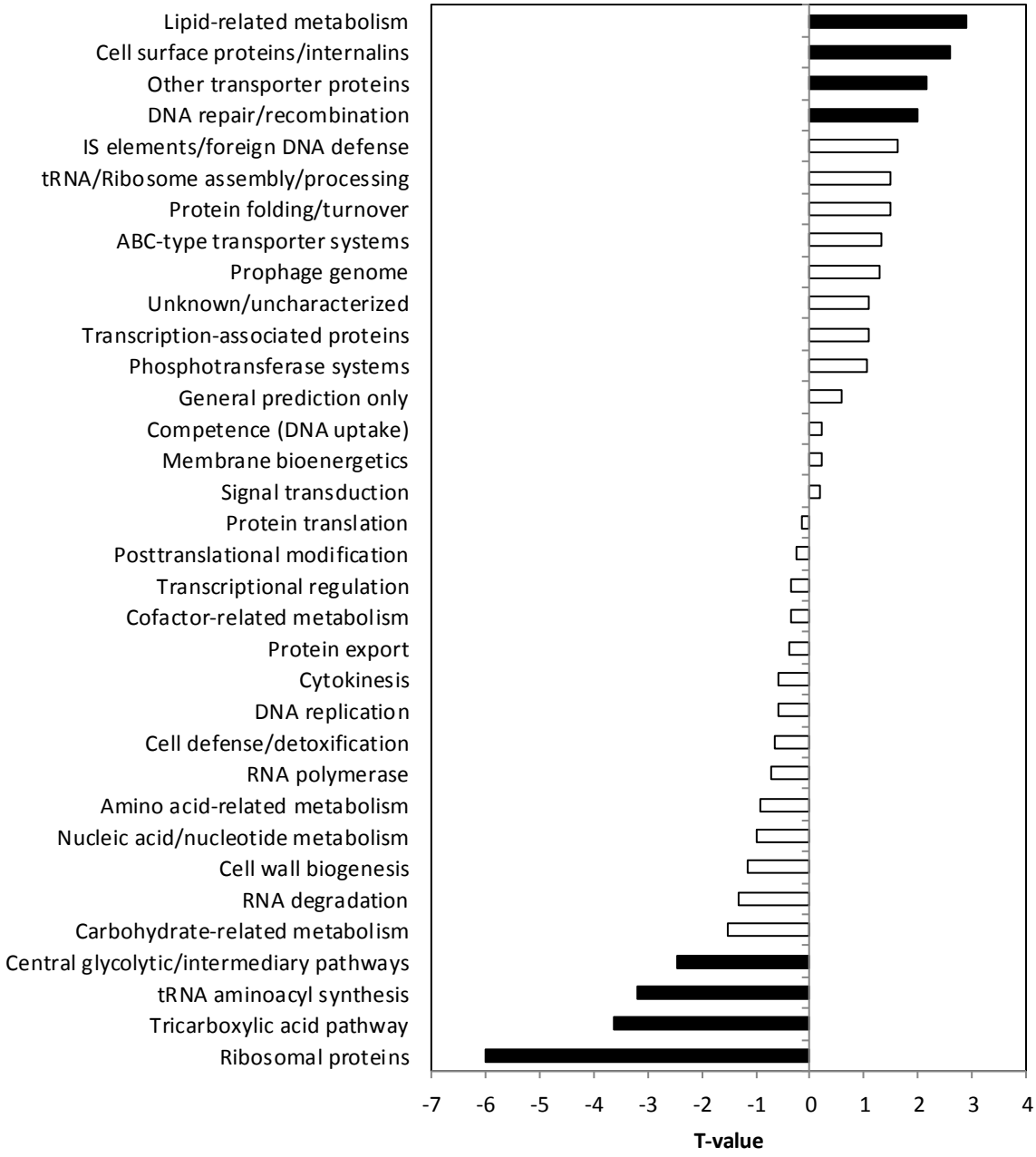


**Figure 3:** Abundance trend of protein functional groups for *Lb. casei* GCRL163.Cells grown on Tween 80 alone in comparison to biomass grown on citrate and Tween 80. Black bars show a significant trend of increased (positive T-values) and decreased (negative T-values) abundance ($p<0.05$).

**Table 2:** Proteins showing alterations of abundance in *Lb. casei* GCRL163 when grown on Tween 80 as a sole carbon source in relation to growth on citrate + Tween 80.

| Equivalent BN194 locus | Gene symbol | Protein Specific Function | Function | fold-change | P |
|---|---|---|---|---|---|
| BN194_28530 | SgaU | L-xylulose 5-phosphate 3-epimerase | ascorbate utilization; Pentose phosphate pathway | 4.51 | 0.018 |
| BN194_06940 | BglP | PTS system beta-glucoside-specific transporter subunit IIBCA | beta-glucoside uptake | 4.27 | 0.009 |
| BN194_10010 | FtsK | DNA translocase FtsK | cell division (chromosomal segegration) | 4.50 | 0.017 |
| BN194_16940 | DnaG | DNA primase | DNA replication initiation | 4.84 | 0.004 |
| BN194_19170 | MecA | negative regulator of genetic competence; adaptor protein | DNA transformation process | 5.41 | 0.131 |
| BN194_03290 | RsbB | ribose ABC-type transport system, substrate=binding protein | D-ribose uptake | 7.13 | 0.003 |
| BN194_23950 | Tmk | thymidylate kinase | dTDP biosynthesis | 6.21 | 0.023 |
| BN194_11050 | | H+ MFS-type efflux transporter | efflux (H+ efflux?) | 4.45 | 0.202 |
| BN194_18780 | YogA | quinone-oxidoreductase | electron transport | 4.51 | 0.018 |
| BN194_21770 | RmlB2 | dTDP-glucose 4,6-dehydratase | exopolysaccharide biosynthesis | 4.01 | 0.466 |
| BN194_22590 | AcpP2 | acyl carrier protein | fatty acid biosynthesis | 35.98 | 0.000 |
| BN194_22580 | FabK | enoyl-[acyl-carrier protein] reductase II | fatty acid biosynthesis | 25.28 | 0.000 |
| BN194_22530 | AccB | acetyl-CoA carboxylase biotin carboxyl carrier protein | fatty acid biosynthesis | 18.48 | 0.001 |
| BN194_22540 | FabF | 3-oxoacyl-ACP synthase I/II | fatty acid biosynthesis | 15.24 | 0.001 |
| BN194_22600 | FabH | 3-oxoacyl-ACP synthase III | fatty acid biosynthesis | 10.62 | 0.006 |
| BN194_22560 | FabG | 3-oxoacyl-ACP reductase | fatty acid | 9.80 | 0.052 |

| | | | | | |
|---|---|---|---|---|---|
| | | | biosynthesis | | |
| BN194_22500-10 | AccC | acetyl-CoA carboxylase biotin carboxylase subunit | fatty acid biosynthesis | 9.76 | 0.020 |
| BN194_22550 | Bkr4 | 3-oxoacyl-[acyl-carrier-protein] reductase 4 | fatty acid biosynthesis | 9.71 | 0.049 |
| BN194_22570 | FabD | 3-oxoacyl-[acyl-carrier-protein] reductase 4 | fatty acid biosynthesis | 8.92 | 0.063 |
| BN194_22810 | Pox5_2 | pyruvate oxidase | fermentation; end-product metabolism | 6.26 | 0.162 |
| BN194_22340 | HsdM | type I restriction modification system, M subunit | foreign DNA restriction | 8.91 | 0.041 |
| BN194_15420 | FruK | 1-phosphofructokinase | fructose catabolism | 10.03 | 0.009 |
| BN194_04030 | | PTS (Fructose/Mannitol family) IIC | fructose uptake | 7.16 | 0.182 |
| BN194_28930 | | PTS (Galacitol family) IIB | galactitol uptake | 5.31 | 0.235 |
| BN194_16280 | HisD | histidinol dehydrogenase | Histidine biosynthesis | 4.50 | 0.017 |
| BN194_02260 | IolD | 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase | inositol-related compound degradation | 4.51 | 0.018 |
| BN194_30430 | | PTS (Fructose/Mannitol family) subunit IIBC | mannitol uptake | 4.48 | 0.014 |
| BN194_25450 | | Mn/Zn ABC transporter substrate-binding protein | Mn/Zn ion uptake | 4.49 | 0.208 |
| BN194_16370 | PanE | 2-dehydropantoate 2-reductase | pantothenate/ CoA biosynthesis | 5.88 | 0.002 |
| BN194_09750 | | phage peptidoglycan hydrolase | phage endolysin | 5.38 | 0.068 |
| BN194_10790 | | phage terminase large subunit | phage terminase | 5.41 | 0.132 |
| BN194_21150 | YjbQ | Trk-type K+ transport ATPase | potassium ion uptake | 4.79 | 0.005 |
| BN194_10170 | SecA | Protein translocase subunit SecA | protein secretion: Sec system (trafficking) | 4.25 | 0.007 |
| BN194_19480 | PrfC | peptide chain release factor 3 | protein synthesis; translation release | 7.10 | 0.186 |
| BN194_21510-20 | TyrS | tyrosine--tRNA ligase | protein synthesis; tRNA charging | 4.74 | 0.116 |

| | | | | | |
|---|---|---|---|---|---|
| BN194_19450 | ClpE | ATP-dependent Clp protease ATP-binding subunit ClpE | recycling defective proteins | 4.28 | 0.008 |
| BN194_21680 | ClpC | ATP-dependent Clp protease ATP-binding subunit | recycling defective proteins | 4.24 | 0.164 |
| BN194_28430 | | LicR family transcriptional activator of PTS | regulation of PTS system genes | 5.35 | 0.134 |
| BN194_27870 | | GntR family transcriptional regulator | regulation of sugar metabolism? | 6.26 | 0.027 |
| BN194_17510 | RibC | riboflavin kinase/FMN adenylyltransferase | riboflavin/flavin mononucleotide metabolism | 5.38 | 0.008 |
| BN194_22030 | | drug resistance MFS-type transporter, EmrB/QacA subfamily | toxic substance efflux | 4.53 | 0.116 |
| BN194_03430 | | transposase, IS5 family | transposition | 4.49 | 0.208 |
| BN194_08380 | Tgt | queuine tRNA-ribosyltransferase | tRNA modification | 7.14 | 0.058 |
| BN194_15470 | Rnz | ribonuclease Z | tRNA processing; RNA degradation | 4.48 | 0.014 |
| BN194_19150 | PyrDA | dihydroorotate dehydrogenase A | UMP/uridine/uracil biosynthesis | 4.50 | 0.017 |
| BN194_25890 | | hypothetical protein BN194_25890 | unknown function | 15.76 | 0.005 |
| BN194_27530 | | hypothetical protein BN194_27530 | unknown function | 9.77 | 0.008 |
| BN194_08830 | | hypothetical protein BN194_08830 | unknown function | 8.86 | 0.000 |
| BN194_16550 | | nitroreductase family protein | unknown function | 7.27 | 0.000 |
| BN194_22260 | | DUF917 superfamily protein | unknown function | 7.10 | 0.002 |
| BN194_06780 | | WxL domain cell surface protein with a lectin binding domain | unknown function | 6.31 | 0.261 |
| BN194_18770 | MqhC | esterase/lipase superfamily protein | unknown function | 6.25 | 0.117 |
| BN194_26590 | | Pip_YghE superfamily membrane spanning protein | unknown function | 6.23 | 0.026 |
| BN194_26450 | | WxL domain cell surface protein with a lectin | unknown function | 5.38 | 0.008 |

| | | | | | |
|---|---|---|---|---|---|
| | | binding domain | | | |
| BN194_22250 | HyuA | D-phenylhydantoinase, hydantoinase superfamily | unknown function | 5.36 | 0.007 |
| BN194_23630 | | hypothetical protein BN194_23630 | unknown function | 5.36 | 0.001 |
| BN194_23980 | | YbaB/EbfC DNA-binding family protein | unknown function | 5.36 | 0.007 |
| BN194_26300-10 | | cell surface protein with repeat Cna_B domains | unknown function | 5.32 | 0.014 |
| BN194_06690 | | hypothetical protein BN194_06690 | unknown function | 4.53 | 0.116 |
| BN194_04050 | | DUF1498 superfamily protein | unknown function | 4.50 | 0.017 |
| BN194_01950 | | alkaline shock protein 23 family protein | unknown function | 4.07 | 0.002 |
| BN194_18970 | YtpR | tRNA-binding protein, tRNA_domain_binding superfamily | unknown function (tRNA-associated?) | 11.48 | 0.000 |
| BN194_26500 | YabR | RNA binding protein, S1_like superfamily | unknown ribosome-associated function | 4.56 | 0.000 |
| BN194_11790 | | two-component system response regulator | unknown signal transduction | 5.36 | 0.007 |
| BN194_01120 | | signaling protein with modified GGDEF and DHH domains | unknown signal transduction | 4.53 | 0.116 |
| BN194_07710 | | ABC-type transporter, substrate-binding protein | unknown transport | 8.88 | 0.007 |
| BN194_02800 | DeoB | phosphopentomutase | (deoxy)ribose 1-phosphate biosynthesis | -14.29 | 0.000 |
| BN194_15250 | RpsT | 30S ribosomal protein S20 | 30S (SSU) ribosome | -4.03 | 0.000 |
| BN194_09950 | RpsN | 30S ribosomal protein S14 | 30S (SSU) ribosome | -4.33 | 0.007 |
| BN194_26090 | RpsZ | 30S ribosomal protein S14 type Z | 30S (SSU) ribosome | -5.49 | 0.039 |
| BN194_17980 | RpmB | 50S ribosomal protein L28 | 50S (LSU) ribosome | -4.29 | 0.111 |
| BN194_26170 | RplV | 50S ribosomal protein L22 | 50S (LSU) ribosome | -5.64 | 0.004 |
| BN194_18800 | RplT | 50S ribosomal protein L20 | 50S (LSU) ribosome | -8.30 | 0.129 |
| BN194_26210 | RplD | 50S ribosomal protein L4 | 50S (LSU) ribosome | -9.00 | 0.061 |
| BN194_18810 | RpmI | 50S ribosomal protein L35 | 50S (LSU) | -45.41 | 0.061 |

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|---|---|---|---|---|---|
| | | | ribosome | | |
| BN194_02120 | CesR | cell wall integrity sensing response regulator CesR | cell wall alteration signalling | -6.33 | 0.061 |
| BN194_20310 | CitF | citrate lyase alpha subunit | citrate utilization | -3.97 | 0.007 |
| BN194_20300 | CitX | apo-citrate lyase phosphoribosyl-dephospho-CoA transferase | citrate utilization | -6.33 | 0.007 |
| BN194_20290 | OadA | oxaloacetate decarboxylase, alpha subunit | citrate utilization | -7.28 | 0.069 |
| BN194_20370 | OadH | oxaloacetate decarboxylase biotin carrier protein | citrate utilization | -7.65 | 0.009 |
| BN194_20320 | CitE | citrate lyase beta subunit | citrate utilization | -10.61 | 0.009 |
| BN194_24320 | | sex pheromone lipoprotein (similar to cAD1 in E. faecalis) | conjugation | -4.29 | 0.004 |
| BN194_30010 | Maa | maltose O-acetyltransferase family protein | detoxification | -7.63 | 0.002 |
| BN194_11560 | GlmS | glucosamine--fructose-6-phosphate aminotransferase | early peptidoglycan biosynthesis | -6.77 | 0.027 |
| BN194_04930 | Sph | fatty acid hydratase/isomerase (oleate hydratase, linoleate isomerase) | fatty acid degradation or detoxification ? | -7.60 | 0.000 |
| BN194_01620 | AckA | acetate kinase | fermentation; end-product formation | -5.59 | 0.002 |
| BN194_16090 | PflA | pyruvate formate-lyase activating enzyme | fermentation; end-product formation | -7.14 | 0.007 |
| BN194_19670 | Pox5 | pyruvate oxidase | fermentation; end-product metabolism | -3.99 | 0.001 |
| BN194_16020 | | aldose 1-epimerase | glycolysis | -5.69 | 0.004 |
| BN194_15110 | PdhD | dihydrolipoyl dehydrogenase | glycolysis | -8.98 | 0.003 |
| BN194_24730 | PpdK | pyruvate, phosphate dikinase | glycolysis | -11.04 | 0.000 |
| BN194_21660 | Fpb | fructose-1,6-bisphosphatase class 3 | glycolysis | -14.32 | 0.016 |
| BN194_07010 | | Cys-based peroxiredoxin, OsmC superfamily | hydroperoxide removal | -9.62 | 0.005 |
| BN194_07500 | LacA | galactose-6-phosphate isomerase subunit LacA | lactose catabolism | -8.34 | 0.001 |

| | | | | | |
|---|---|---|---|---|---|
| BN194_16640 | LplJ | lipoate-protein ligase LplJ | lipoate metabolism | -10.17 | 0.002 |
| BN194_24910 | ProB | glutamate 5-kinase | L-proline biosynthesis | -4.33 | 0.018 |
| BN194_08130 | YjbG2 | oligoendopeptidase F | peptidase for amino acid acquistion | -5.92 | 0.000 |
| BN194_08940 | | phosphatidylglycerophosphatase A | phospholipid degradation | -6.30 | 0.000 |
| BN194_11290 | PotA | spermidine/putrescine import ATP-binding protein PotA | polyamine uptake? | -4.29 | 0.000 |
| BN194_23520 | Ptp | tyrosine-protein phosphatase | protein phosphorylation (regulation)> | -10.29 | 0.008 |
| BN194_20080 | SerS | serine--tRNA ligase | protein synthesis; tRNA charging | -5.22 | 0.000 |
| BN194_19410-20 | PtsI | phosphoenolpyruvate-protein phosphotransferase | PTS general components | -8.95 | 0.001 |
| BN194_02810 | DeoD | purine nucleoside phosphorylase | purine nucleoside interconversion/salvage | -8.94 | 0.000 |
| BN194_08750 | CcpA | catabolite control protein A | regulation of catabolic enzyme coding genes | -7.50 | 0.000 |
| BN194_25440 | CzrA | ArsR family transcriptional repressor | regulation of copper resistance | -4.29 | 0.001 |
| BN194_17060 | YqfL | phosphotransferase activator of gluconeogenesis | regulatory role (gluconeogenesis?) | -4.08 | 0.000 |
| BN194_22870 | | universal stress protein, UspA superfamily | stress regulation? | -4.40 | 0.001 |
| BN194_24800 | | CsbD superfamily protein | stress response (phosphate starvation)? | -4.33 | 0.000 |
| BN194_11260 | UgpB | glycerol-3-phosphate ABC transporter substrate-binding protein | sugar/glycerol uptake | -4.50 | 0.000 |
| BN194_15170 | Pyc | pyruvate carboxylase | TCA cycle | -6.87 | 0.040 |
| BN194_21400 | GalE2 | UDP-glucose 4-epimerase | teichoic acid decoration | -10.92 | 0.015 |
| BN194_02420 | | glycosyltransferase family | teichoic acid | -4.31 | 0.002 |

| | | | | | |
|---|---|---|---|---|---|
| | | protein | decoration? | | |
| BN194_07650 | ThiD2 | Hydroxymethylpyrimidine/ phosphomethylpyrimidine kinase | thiamine biosynthesis | -8.30 | 0.001 |
| BN194_02070 | EngD | GTP-and nucleic acid-binding protein | translation attenuation? | -6.92 | 0.002 |
| BN194_27510 | | aldo_keto_reductase superfamily protein | unknown function | -3.96 | 0.003 |
| BN194_17960 | | Dak2 domain fusion protein | unknown function | -4.17 | 0.002 |
| BN194_05660 | | DUF1883 superfamily protein | unknown function | -4.31 | 0.000 |
| BN194_20400 | | hypothetical protein BN194_20400 | unknown function | -4.99 | 0.000 |
| BN194_19080 | | DUF964 superfamily protein | unknown function | -5.00 | 0.006 |
| BN194_30450 | | hydrolase, HAD superfamily | unknown function | -5.66 | 0.000 |
| BN194_28520 | YidA | hydrolase, HAD superfamily | unknown function | -5.73 | 0.001 |
| BN194_08670 | YkuL | CBS_pair superfamily protein | unknown function | -7.00 | 0.006 |
| BN194_07660 | | hypothetical protein BN194_07660 | unknown function | -7.22 | 0.000 |

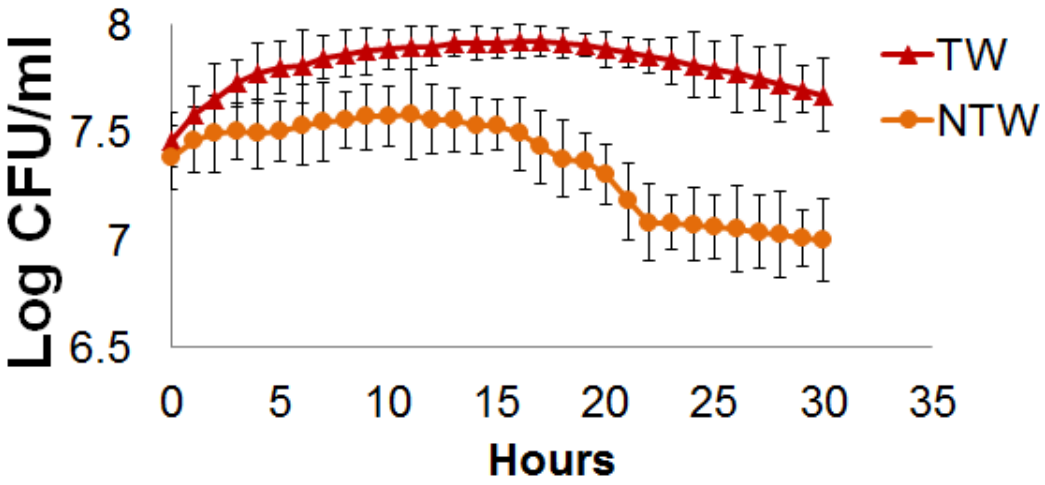


**Figure 4:** Growth of *Lb. casei* GCRL163 in modified buffered MRS broth at pH 2.5 in the presence or absence of Tween 80. TW: Tween 80, NTW: No Tween 80.

**Tween 80 influences lipid metabolism and enhances acid resistance.** A stark effect was observed on fatty acid biosynthesis pathway with the primary pathway enzymes increased substantially in abundance (Figure 2) during sole growth on Tween 80. In chapter 2 it was observed lactose starved cells forced to grow on citrate had suppressed fatty acid biosynthesis enzymes (AcpP2, FabF, FabG, Cfa) relative to those grown without Tween 80. This suggests the metabolism of Tween 80 results in regulatory changes that could affect the activity of fatty acid biosynthesis, apparently more active in Tween 80 grown cells in a relative sense. Auxiliary enzymes in the pathway, including cyclopropane fatty acid synthetase exhibit a slight increase in abundance (Table 2). This suggests that fatty acid synthesis itself occurs more prominently but compositional change is not confirmed. It has been noted that Tween 80 supplementation affects fatty acid profiles including higher levels of oleic acid and cyclopropane fatty acid (Wu *et al*., 2013; Broadbent *et al*., 2010), when cells are exposed to acidic conditions. The results here thus strongly suggest that fatty acid turnover is increased in the presence of Tween 80 compared to when citrate is also present.

Responses to Tween 80 are known to be strain variable thus the effect on Tween 80 on fatty acid composition may only be one aspect of its effect on cells that can affect stress responses. It was confirmed that when GCRL163 was exposed to a lethal pH challenge (pH 2.5 for >30 h) that Tween 80 provides substantially enhanced survival (Figure 4). This enhanced survival has been indicated to be due to Tween 80's effect on the cell membrane, reducing the entry of $H^+$. The effects of Tween 80 fatty acid biosynthesis appears logically connected. However it also possible Tween 80 in its role as a potential inhibitor, which it achieves by its surfactant properties, could cause membrane disturbance (Oh *et al*., 1995) and induce stress responses in cells. For example, Tween 80 at only 20 ppm (50-times lower than the level used in this study) has been shown to inhibit marine bacteria, mainly Gram-negative

species (Buck and Cleverdon 1961). Thus it can be assumed an outcome of Tween 80 exposure leads to stress responses that could subsequently manifest as “hardening” against others forms of stress. An apparent increase in the abundance of the fatty acid biosynthesis enzymatic pathway could be associated with increased fatty acid turnover overcoming disruption effects of the Tween 80. Since citrate is a better energy source it could be suggested that the effect of Tween 80 could be due to a lower availability of acetyl-CoA. This can be supported by the observation that the pyruvate dehydrogenase complex (which converts pyruvate to acetyl-CoA) is less active in Tween 80 grown cells with abundance reduced for all subunits, especially PdhD (which has dihydrolipoyl dehydrogenase activity) reduced 9-fold (Table 2). Further metabolite analysis and enzyme studies are needed to further explore Tween 80 responses to further clarify these changes.

**Table 3:** Proteins associated with stress responses in *Lb. casei* GCRL163 that are more abundant when grown on Tween 80 as sole carbon source.

| Equivalent BN194 locus | Protein symbol | Function | Function | Fold-change | P |
|---|---|---|---|---|---|
| BN194_00680 | OpuCA | glycine betaine/carnitine/choline ABC-type transporter, ATP-binding protein | carnitine/choline/ glycine betaine uptake | 7.11 | 0.058 |
| BN194_17950 | RecG | ATP-dependent DNA helicase RecG | DNA repair (homologous recombination) | 4.51 | 0.018 |
| BN194_08360 | RuvB | Holliday junction resolvasome, helicase subunit | DNA repair (homologous recombination) | 4.48 | 0.014 |
| BN194_25470 | | lactaldehyde dehydrogenase/glycolaldehyde dehydrogenase | detoxification of aldehydes | 6.24 | 5.78E-08 |
| BN194_23760 | GroES | class I heat-shock protein (chaperonin) small subunit | nascent protein folding | 5.17 | 0.001533 |
| BN194_04740 | Npr | NADH peroxidase | oxidative stress management | 6.02 | 0.00041 |
| BN194_29780 | YecS | amino-acid ABC-type importer substrate-binding/permease components | polar amino acid uptake | 4.53 | 0.116 |
| BN194_29910 | GlnH4 | polar amino acid ABC-type importer, permease component | polar amino acid uptake | 4.51 | 0.018 |
| BN194_29920 | GlnH5 | polar amino acid ABC-type importer, permease component | polar amino acid uptake | 4.48 | 0.013915 |
| BN194_24060 | | PII-type proteinase | protein degradation (casein) | 6.81 | 0.023 |
| BN194_29440 | | alpha-crystallin domain heat shock protein | protein disaggregation (during rapid growth) | 33.3 | 5.51E-06 |
| BN194_07570 | Hsp18 | alpha-crystallin domain heat shock protein | protein disaggregation (during rapid growth) | 6.20 | 0.116 |
| BN194_06990 | Csp | Cold shock protein 1 | RNA chaperone | 7.57 | 4.23E-05 |

| | | | | | |
|---|---|---|---|---|---|
| BN194_12460 | CspLA | Cold shock-like protein CspLA | RNA chaperone | 4.08 | 0.0012 |
| BN194_19450 | ClpE | ATP-dependent Clp protease ATP-binding subunit ClpE | recycling defective proteins | 4.28 | 0.0079 |
| BN194_21680 | ClpC | ATP-dependent Clp protease ATP-binding subunit | recycling defective proteins | 4.24 | 0.164 |

**Tween 80 and stress response induction in *Lb. casei* GCRL163.** There is also increased abundance of cell surface proteins including those putatively involved in adhesion and lectin-binding as well as others with more mysterious roles. Tween 80 tends to reduce cell clumping, as observed during culture of GCRL163 without Tween 90, suggesting a decreased cell surface charge. This change may correspond to a general increase in the abundance of cell surface proteins (Figure 2). Assessment of the proteome data also indicated several general responses relevant to stress adaptation summarized in Table 3. These responses may arise from the metabolic needs of catabolizing Tween 80 since they seem largely non-specific. Increases in proteins involved in nascent protein folding (GroES), turnover (ClpC, ClpE and disaggregation (Hsp18) (Spano *et al*., 2005) could be protective in the situation where cytoplasmic pH declines as suggested in the acid tolerance experiment (Figure 4). This is supported by increases in transporters for polar amino acids (Wu *et al*. 2013; Broadbent *et al*. 2013) and compatible solutes that contribute to intracellular protein integrity and acid tolerance adaptation, which is discussed more in Chapter 4. Other responses such as increases in abundance of DNA repair enzymes RecG and RuvB, cold shock proteins, and NADH oxidase, the latter potentially involved in removal of $H_2O_2$ formed during pyruvate oxidation.

In conclusion, the overall data suggests that the GCRL163 can grow directly on Tween 80 possibly attacking the sorbitan section of the molecule as well as utilizing the oleic acid component of the molecule for energy production. Tween 80 has been incorporated into growth media of many species of LAB for a considerable period (see Williams *et al*., 1947), where it improves growth, obviates the need for supply of essential vitamins, such as biotin, and provides a source of unsaturated fatty acids (specifically oleic) which are essential for some LAB species for growth (Partanen *et al*., 2001). Early studies on the impact of Tween

compounds and fatty acids on growth used media similar to MRS but generally incorporated sugars as carbon sources and used short incubation periods (usually up to 72 h), so that growth on Tween 80 as a main carbon source remained undetected although impacts on the degree of growth and provision of saturated fatty acids or vitamin substitutes was well documented. The results reported in this work clearly show that Tween 80 can be metabolized when other carbon sources are not available and can provide all cellular needs, although growth is clearly under stress. Tween 80's ability to enhance the survival of GCRL163 can be linked to a broad induction of stress responses in the cell beyond that related to fatty acid metabolism. Proteomic data also suggests Tween 80 changes the surface properties of GCRL163 potentially linked to markedly more abundant cell surface proteins. The data shown here contributes to explaining the effect of Tween 80 on the physiology of GCRL163. Application of Tween 80 or other lipid molecules could be a useful means to adjust probiotic physiological properties enhancing survival in food or non-food delivery systems.

# CHAPTER FOUR

# Growth phase dependent and independent acid adaptation responses in *Lactobacillus casei*


## Abstract

Acidic environments in food products affect the survival and therefore the potential efficacy of probiotic bacteria. In this study, acid stress adaptation responses of *Lactobacillus casei* strains, including fermented milk strain MJA12 and Cheddar cheese isolate GCRL163, were investigated using label-free quantitative proteomic analysis of cell-free lysates. The strains were grown under anaerobic conditions in MRS broth adjusted to and maintained at pH 4.5 or pH 6.5 in fermenters, with biomass collected during mid-exponential and early-stationary growth phases. Approximately 35% of the proteome of each strain was identified to >95% confidence. Distinct patterns and responses were observed from the proteome data: i) proteome level change at pH 4.5 relative to pH 6.5 were similar to that observed between stationary growth phase and exponential growth phase cultures at pH 6.5; ii) both strains exhibited increased abundance of olipopeptide/dipepetide importers, peptidases/proteinases, and branched chain aminotransferase,and iii) increases in the abundance of proteins in the fatty acid biosynthetic pathway and enolase during active growth was strain-dependent. These results are consistent with the main means by which *Lb. casei* combats sudden acid stress, including exogenous amino acid accumulation, and increased unsaturation of fatty acid. Protein abundance data, however, revealed that acid adaptation varied between the strains in a growth phase-dependent manner, with strain MJA12 enhancing known acid adaptation-type responses when in the stationary growth phase, unlike strain GCRL163 which appeared to be more directly responsive to acidic pH conditions, adapting during exponential growth. Furthermore, growth at low pH resulted in strain MJA12, but not strain

GCRL163, producing increased levels of malolactic fermentation associated enzymes, primarily malic enzyme MleA. Differences in the proteomes suggest that adaptation to growth at low pH varies between *Lb. casei* strains and thus strains are likely to react and persist differently in food and host systems.

## Introduction

*Lactobacillus casei* is an acid-resistant, Gram-positive, rod-shaped, heterofermentative bacterial species included in the broad group referred to as the lactic acid bacteria (LAB). *Lactobacillus* species can be found in or on fresh or fermented food products including vegetables, fruit, milk and meat, where they can contribute to food organoleptic properties (Law Ba, 2001; Smit *et al*., 2001; Hufner *et* al., 2007; Bisanz and Reid., 2011). Like other LAB, *Lb. casei* produces lactic acid as its major end product of carbohydrate fermentation. During fermentation *Lb. casei* exports lactic acid and $H^+$ out of the cytoplasm in order to control the internal pH ($pH_i$) and generate ATP, resulting in acidification of the surrounding medium (Booth 1985). Aciduricity is the ability of the bacteria to grow and survive in acidic, organic acid-rich conditions. This ability is an important criterion when selecting probiotic strains that can be delivered in functional foods (Cotter and Hill, 2003; Bisanz and Reid, 2011; Wu *et al*., 2011). The understanding of physiological aspects connected to acidiuricity may better enable strain selection and application of probiotics via food delivery systems.

When under acidic conditions, lactobacilli can tolerate a $pH_i$ decline to a certain extent (Shabala *et al*., 2006), unlike many other bacteria which usually rigorously maintain cytoplasmic pH homeostasis around neutral. Lactobacilli usually lack metabolic-based pH homeostasis mechanisms, such as the glutamate decarboxylase and the arginine deaminize

systems, and rely on $F_1F_0$-ATPase to aid in $pH_i$ homeostasis via $H^+$ extrusion (Cotter *et al*., 2003). Under acid shock, *Lb. casei* has been shown to modulate fatty acids with changes in cyclopropane fatty acid content, oleic acid (18:1ω9c) ratios and fatty acid acyl chain length increases, which is understood to be linked with increased acid resistance (Wu *et al*., 2012). These changes presumably lead to reduced permeability to $H^+$ and organic acids (Wu *et al*., 2012). L-malate, L-histidine (Broadbent *et al*., 2010) and L-aspartate (Wu *et al*., 2013) supplied exogenously were found to protect cells against acid shock, presumably contributing indirectly to $pH_i$ homeostasis.

The aim of the research described here was to investigate the proteomes of two *Lb. casei* grown under neutral and acidic conditions for cells harvested at different growth phases in order to understand the nature of acid adaptation, considering strain and growth phase variables. The strains investigated were GCRL163, a Cheddar cheese isolate (Chandry *et al*., 1998) and MJA12, isolated from the fermented milk product Yakult™ (Yakult Honsha Co., Ltd), which contains a probiotic *Lb. casei* culture. The proteomes of cell-free lysates were determined using a gel-free, label-free approach with quantification of protein abundance in relative terms employed between treatments (Al-Naseri *et al*., 2013). The information, when summarised at both specific and broad functional levels, demonstrates that, besides known acid resistance mechanisms, there are additional parameters related to strain type and growth phase that influence acid adaptation.

**Material and methods:**

**Bacterial strain and growth conditions.** *Lb. casei* strain GCRL163, a Cheddar cheese isolate, has been described previously (Chandry *et al*., 1998; Al-Naseri *et al*., 2013). Strain MJA12 was isolated in the present study from Yakult™ milk drink (Yakult Honsha

Co., Ltd) following culture and purification on MRS agar. Both strains were confirmed as *Lb. casei* by 16S rDNA sequencing (Chandry *et al*., 1998) and stored as described previously (Al-Naseri *et al*., 2013). Multilocus sequence typing analysis (MLST) was undertaken as described by Diancourt *et al*. (2007) using seven sequence alleles for housekeeping genes (*fus*, *ileS*, *leuS*, *lepA*, *pyrG*, *recA*, *recG*) and results matched against the Pasteur Institute *Lactobacillus casei* MLST Database. (www.pasteur.fr/recherche/genopole/PF8/mlst/Lcasei.html).

Strains were revived from glycerol storage by passaging through two sequential subcultures in MRS broth (Oxoid, Australia) incubated at 37 °C for 12 h under anaerobic conditions (Anaerocult A system, Oxoid, Australia). Cultures were plated onto MRS agar and incubated anaerobically at 37 °C for 48 h then starter cultures prepared by suspending a single colony of each strain into MRS broth. One individual colony represented one biological replicate. Each stationary-phase starter culture was used to inoculate (2% v/v) 900 ml of MRS in Bioflo/Celligen 115 Benchtop Fermentor/Bioreactor systems (New Brunswick, USA). Prior to inoculation, media were sparged with nitrogen gas to maintain strictly anaerobic conditions and $O_2$ levels monitored during culture using a dissolved $O_2$ probe, with gas addition controlled during removal of samples. Temperature of bioreactors was controlled at 30 °C with stirring at 100 rpm. As required, the initial pH of media was adjusted with HCl and subsequently controlled automatically during culture through the addition of 2 M HCl and 2 M NaOH.

**Growth at low pH.** To determine the maximum specific growth rate ($\mu_{max}$) at different initial set pH values, MRS broths were prepared in duplicate sets of bioreactors with initial pH of 2.5, 4, 4.5, 5, 5.5, 6.0 or 6.5, inoculated as above and growth monitored by

measuring optical density at 600 nm ($OD_{600}$) and determining viable counts on MRS agar; strains were grown at pH 6.5 or 4.5, 30 °C, overnight before being inoculated into corresponding duplicate fermenters. To evaluate proteomic changes in response to growth at low pH, two sets of three bioreactors at pH 4.5 or 6.5 were inoculated, growth monitored and cells harvested during exponential and stationary growth phases: this corresponded to 8 and 20 hours culture at pH 4.5 and 5 and 10 hours at pH 6.5. Samples (150 ml) of culture broth were centrifuged at 5,000 rpm for 10 min at 4 °C using (Avanti J-30I High-Performance Centrifuge, Beckman, USA), and the cell pellets were washed three times with Tris-HCl buffer (40 mM, pH 7.0). The cells were resuspended in Tris-HCl buffer (40 mM, pH 7.0), concentrating to a standard $OD_{600}$ equivalent to 10. Each bioreactors sample represented a single biological replicate.

**Protein extraction and peptide sample preparation.** Protein was extracted by bead beating 200 µl of cell suspension with 0.5 g of zirconia-silicon beads (0.1 mm diameter; Daintree Scientific, Australia) using a Tissuelyser II bead beater (Qiagen) at 30 cycles/s for 6 min. Cellular debris was removed by centrifugation at 14,000 rpm (Eppendorf | 5417R | Refrigerated Centrifuge, Australia) for 30 min at 4 °C. Peptide samples were prepared by co-precipitation of proteins with trypsin at a substrate: enzyme ratio of 50:1. Protein samples were alkylated by incubation with 50 mM iodoacetamide in the dark at ambient temperature for 2 h. Proteins were co-precipitated with 1 µg proteomics grade trypsin (Sigma) using 1 ml of methanol (overnight at -20 °C). After centrifugation, protein-trypsin precipitates were carefully resuspended in 100 mM ammonium bicarbonate and trypsin digestion allowed to proceed at 37 °C for 5 h. Digests were terminated by addition of 0.1% formic acid and non-digested protein was removed by centrifugation 13,000 rpm using (Eppendorf | 5417R | Refrigerated Centrifuge, Australia) for 5 min (Al-Naseri *et al*., 2013).

**Peptide analysis by LTQ-Orbitrap mass spectrometry and protein identification.** Nano LC and MS/MS analysis was performed using an LTQ-Orbitrap XL and Surveyor autosampler and MS Pump Plus controlled by Xcalibur 2.0 software (Thermo Scientific). Peptides were separated and analysed with minimal void volume LC system using two PEEK Zero Dead Volume (ZDV) tees installed either side of a sample trap column assembly (Upchurch) loaded with a 2.5 cm x 75 μm ID IntegraFrit trapping column (New Objective). Approximately 2 μg of peptide sample was loaded onto the trapping column using 100% mobile phase buffer A (0.1% formic acid) at a flow rate of 20 μl/min over 5 minutes. During sample loading, solvent and unbound analyses were diverted to waste via the downstream ZDV tee. Flow rate was then split down to 200 nl/min using the upstream ZDV tee and peptides were eluted onto a 10.5 cm analytical nanoLC column (New Objective) using a four-step gradient of 100% buffer B (90% acetonitrile in 0.2% formic acid) to 90% buffer C (90% acetonitrile in 0.2% formic acid). Data-dependent tandem mass spectrometry and data acquisition parameters were essentially as described previously (Al-Naseri *et al*., 2013).

**Database searching and criteria for protein identification**. The MS/MS data extracted from the raw files were searched against the combined complete proteomes of strains ATCC 334 (Makarova *et al*., 2006), BL23 (Maze *et al*., 2010), LC2W (Chen *et al*., 2011), BDII (Chen *et al*., 2011), Zhang (Zhang *et al*., 2010), and W56 (Hochwind *et al*., 2012) since they effectively cover a substantial proportion of genetic diversity within the *Lb. casei* The database was downloaded on 26/03/2013 and comprised 6126 entries.

**Statistical analysis of MS/MS data.** The spectral count (SpC), a sampling statistic output of ProteinProphet, was used to determine relative protein abundance (Nesvizhskii *et al*., 2003; Gokce *et al*., 2011). To account for sampling depth the approach of (Gokce *et al*.

2011) was used, in which the overall average of the total SpC for each replicate was used to normalize each SpC value. The variation between each replicate compared to the average total SpC was -11% to +8%. $Log_2$-fold changes in protein expression under each treatment condition were calculated based on the average SpC and a pseudospectral count value of 0.5 was applied to each replicate to allow for comparisons where proteins were not detected in particular treatments (Anderson and Willis, 2003). Significant differences between SpC values were determined using *t*-tests since SpC data was determined to be log-normally distributed, according to the Shapiro-Wilk normality test. Significant alteration in protein abundance was considered to comprise a $log_2$-ratio change of >1 or <-1 (*P*-value of <0.05) thus moderating unexpectedly high *t*-statistic scores.

**Multivariate statistical comparisons**. Due to the complexity of the dataset, the derived protein profiles for each replicate and factors (strain, pH, growth phase) were assessed initially with multivariate statistics using Primer-6 software (Primer-E, Plymouth, UK). This followed the approach of Anderson and Willis (2003) where firstly treatment/strain-associated datasets were compared without constraints using multi-dimensional scaling analysis followed by a constrained analysis utilizing canonical analysis of principal coordinates (CAP), in which each of the treatment criteria were considered fixed variables. For this purpose the SpC data was log(X+1) transformed and converted to Bray-Curtis similarity coefficients (to scale between 0 to 100). The influence of strain, growth phase and pH on the protein profiles was assessed using permuted multivariate analysis of variance (PERMANOVA). The analysis was done using an unrestricted permutation of the data (n=9999), fixed terms summed to zero, and utilizing partial sum of squares since the data is effectively an unbalanced data layout with proteins frequently not observed owing to detection limitations and physiological effects.

**Protein functional group analysis.** The effects of acidic pH and comparison of the change from exponential to stationary growth phase was assessed on a functional level as done by Liu *et al*. (2004) and Bowman *et al*. (2012). For this purpose identified proteins were assigned to a functional category on the basis of gene ontology. Changes in protein abundance within these sets, in relation to the entire dataset, were tested using a penalized *t*-statistic approach devised by Boorsma *et al*. (2005), developed originally for cDNA microarray data. For this analysis the proteins considered included only those that were detected. The derived functional groups *T*-values for each treatment comparison were compared collectively in heat maps by utilizing unsupervised hierarchical cluster analysis. Clustering of *T*-values was performed with Cluster 3.0 (De Hoon *et al*., 2004) using uncentered correlation and complete linkage analysis. The heat map, which visualizes the collected *T*-value data, was constructed in Java Tree View (Saldanha *et al*., 2004).

## Results

Both strains were grown anaerobically in MRS broth constantly maintained at pH 6.5 and pH 4.5 in bioreactors and so they were exposed to a combination of acidity in the form of $H^+$ and unprotonated sodium acetate (33.5 mM) in MRS in addition to end-product accumulation (mainly lactate). Thus it can be assumed that physiological adaptation to acidic conditions incorporates a response to both elevated $H^+$ ions and organic acids.

**Proteome coverage of *Lactobacillus casei* strains investigated.** The strains tested were chosen on the basis that they have a capacity to grow, survive, and are effectively delivered in acidic food types. MLST analysis indicated MJA12 and GCRL163 belonged to sequence types 1 and 2, respectively, within the Pasteur Insititute MLST scheme thus are genetically different though it is assumed a high proportion of their proteomes are highly

similar (Douillard *et al*., 2013). Across all treatments, a total of 1154 proteins were identified to a confidence level of >95%. Of these, 925 and 844 proteins had at least two unique peptides amongst those identified in GCRL163 and MJA12, respectively (Appendix B). The data primarily reflects cytosolic-associated proteins due to inherent extraction biases against proteins with high GRAVY indexes, those that are sorted and covalently linked to peptidoglycan, and those that are mostly secreted extracellularly. Nevertheless, the entire suite of functional protein groups is observable and the data provides a clear indication of housekeeping and metabolic priorities on the basis of relative abundance of individual proteins. The most abundant protein detected was elongation factor Tu (TufA), which is consistent with its central role in protein translation.

**Protein profile comparisons using CAP and functional group analysis reveal divergent responses of *Lactobacillus casei* strains to acid growth.** Despite growth rates and yields of both strain being similar at pH 4.5 (0.47-0.49 $h^{-1}$ ), the maximum specific growth rate responses across a range of pH do not fully align (Figure 1) suggesting potentially strain-specific differences may occur during acid adaptation. Broad appraisal of the proteomic data using CAP analysis (Figure 2) showed surprising distinctiveness and complexity in the responses to acidic conditions. The starkest observation is that strain GCRL163 shows considerably greater divergence in its proteomes at pH 4.5 for both growth phases compared to that of MJA12 in relation to proteomes derived from biomass grown at pH 6.5, where the strains are comparatively more similar. It could be readily discerned that in order of influence on the proteomes growth phase is the most influential factor followed by strain and then by pH conditions, based on the components of variance derived from the PERMANOVA analysis (Appendix C). All factors were significantly different ($p<0.0001$), the distinctiveness enhanced by the fact that biologically-derived variation was quite restricted.

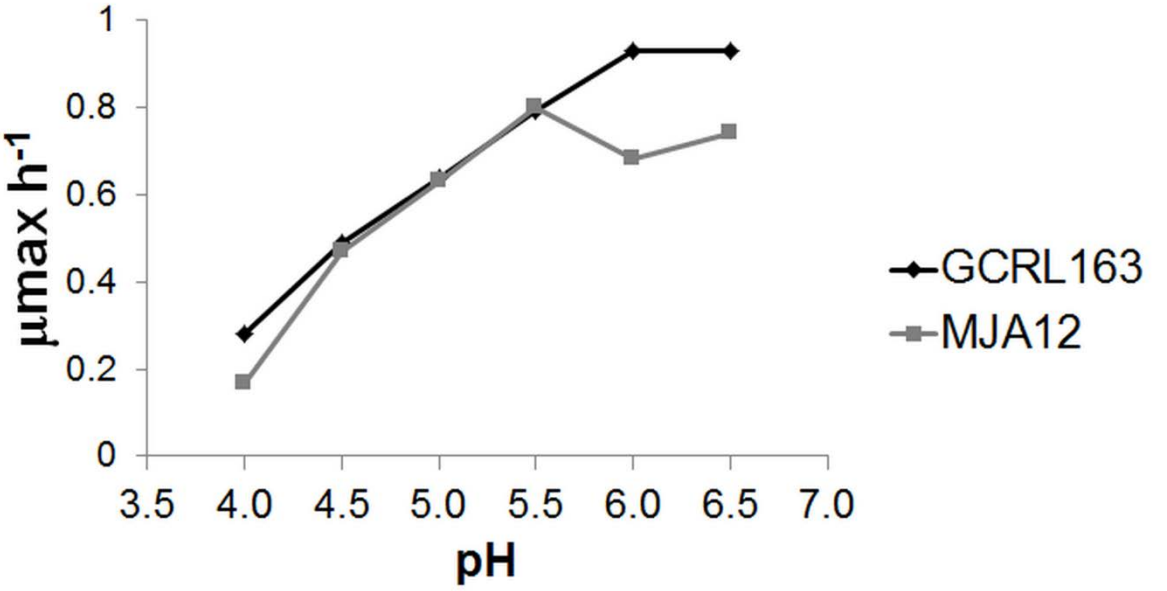


**Figure 1.** Maximum specific growth rate of *Lactobacillus casei* GCRL163 (—◆—) and MJA12 (—■—) in MRS broth under anaerobic conditions with pH maintained at pH 4, 4.5, 5, 5.5, 6, and 6.5.

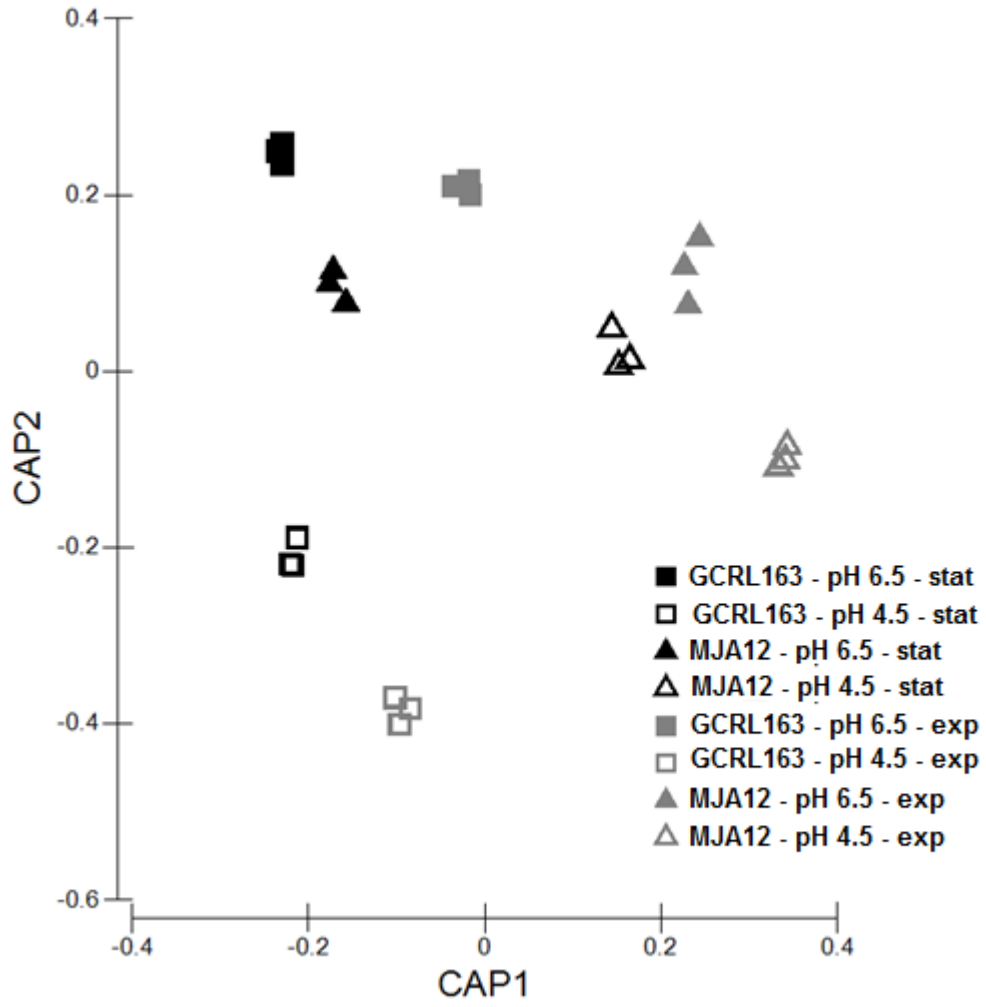


**Figure 2.** Demonstrating the comparison of protein profiles for each replicate and factors (strain, pH, growth phase) utilizing canonical analysis of principle coordinates (CAP).

Hierarchical cluster analysis of functional data supported the CAP analysis in that it demonstrates divergence of proteomes in terms of acid growth adaptation is dependent on a combination of growth phase specific-changes that are non-congruent between the strains tested (Figure 3). The cluster data for GCRL163 suggests the acid-adapted state acquired during active (exponential) growth is broadly similar to that acquired during stationary growth phase when pH is maintained deliberately at pH 6.5. On the other hand strain MJA12 exhibits functionally different trends to GCRL163 during active growth at pH 4.5 but consistently also showed that the acid adapted state is relatively similar to that found for stationary growth phase biomass that was kept under neutral pH conditions. Similarly, proteome divergence is observed between both strains for biomass collected during stationary growth phase when maintained at pH 4.5, which reflects a more natural situation of organic acid accumulation during fermentation.

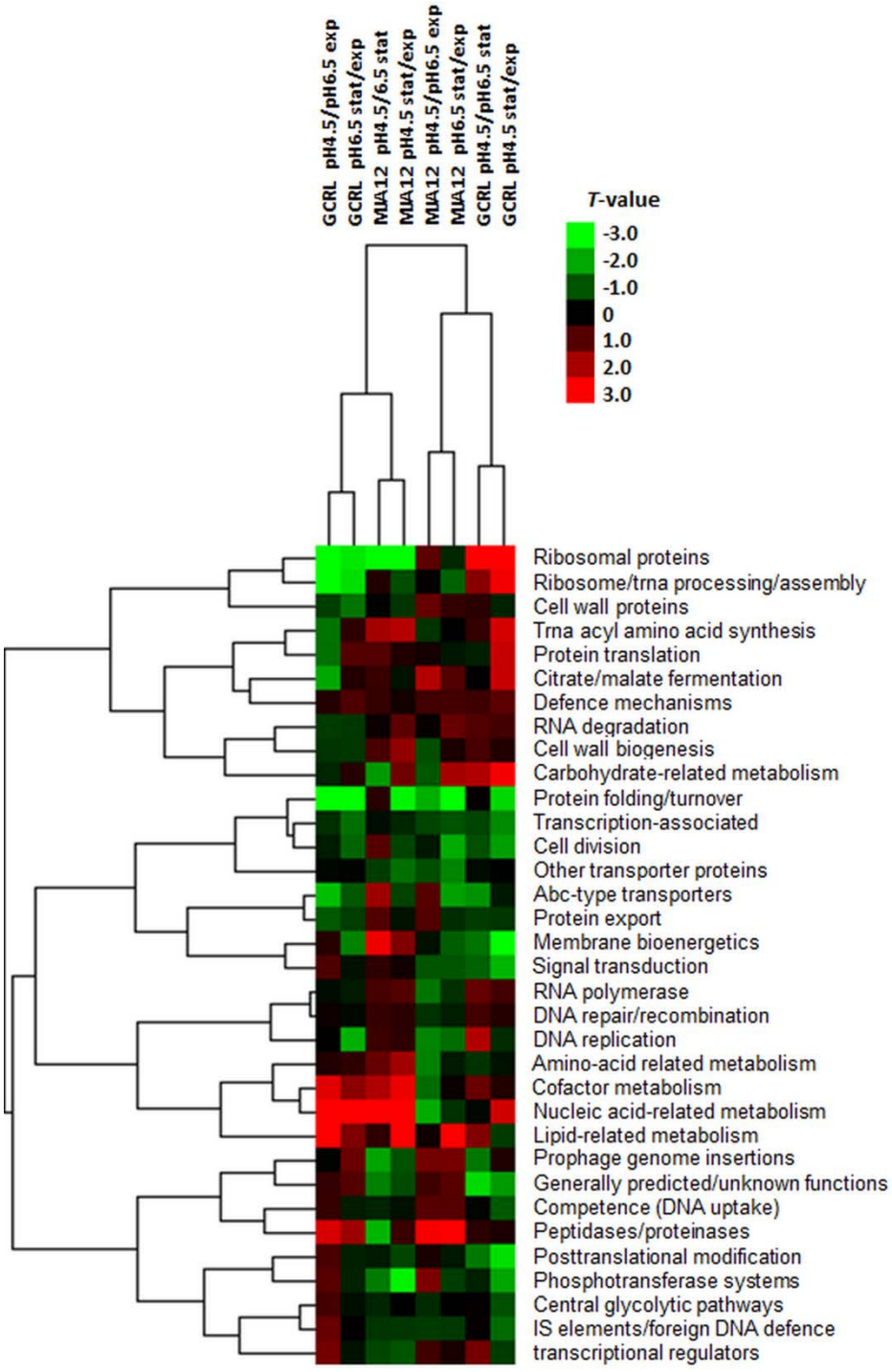


**Figure 3.** Heat map generated from *T-value* data organized by functional groups using Cluster v. 3.0.38. Unsupervised hierarchical clustering on the basis of uncentered correlation was used to compare the overall proteome data sets (top dendrogram) and the protein functional groups (left-handendrogram). G: *Lb. casei* GCRL163; S: *Lb. casei* MJA12; exp: Exponential growth phase; stat: Stationary growth phase.

**Fatty acid biosynthesis proteins and fatty acid hydratase increase in abundance during acid stress while cyclopropane fatty acid synthetase is abundant under all growth conditions.** Most enzymes that make up the fatty acid biosynthesis pathway showed greater abundance in strain GCRL163 when it was exponentially growing at pH 4.5 compared to near neutral pH conditions. The abundance of the pathway enzymes was also elevated in stationary growth phase independent of pH (Figure 4). Surprisingly, strain MJA12 had only weak or undetectable abundance of fatty acid biosynthetic enzymes during active growth at either pH (Table 1, Appendix C), however during stationary growth phase the abundance profile was similar to GCRL163 (Figure 4). The relative abundance of cyclopropane fatty acid synthetase (Cfa) in both strains was not significantly altered by either pH or growth phase suggesting it is constitutively active at all stages of growth. In GCRL163 the abundance of 3-oxoacyl-ACP synthase I/II (FabF) involved in initiating nascent fatty acid acyl chain synthesis was increased substantially only under acidic conditions, while in MJA12 FabF was detectable at both pH 4.5 and 6.5 in the stationary growth phase. This suggests a difference in the way fatty acid content is modulated between the strains. A similar result was also obtained for a protein designated Sph, typically annotated on genomes as myosin cross-reactive antigen, has been shown to be a FAD-dependent fatty acid hydratase (Volkov *et al*., 2010; Volkov *et al*., 2013) involved in fatty acid desaturation. This result is consistent with observations that unsaturated fatty acids become elevated after *Lb. casei* is exposed to an acidic shock (Wu *et al*., 2012), however growth phase status could be important in dictating strain survival.

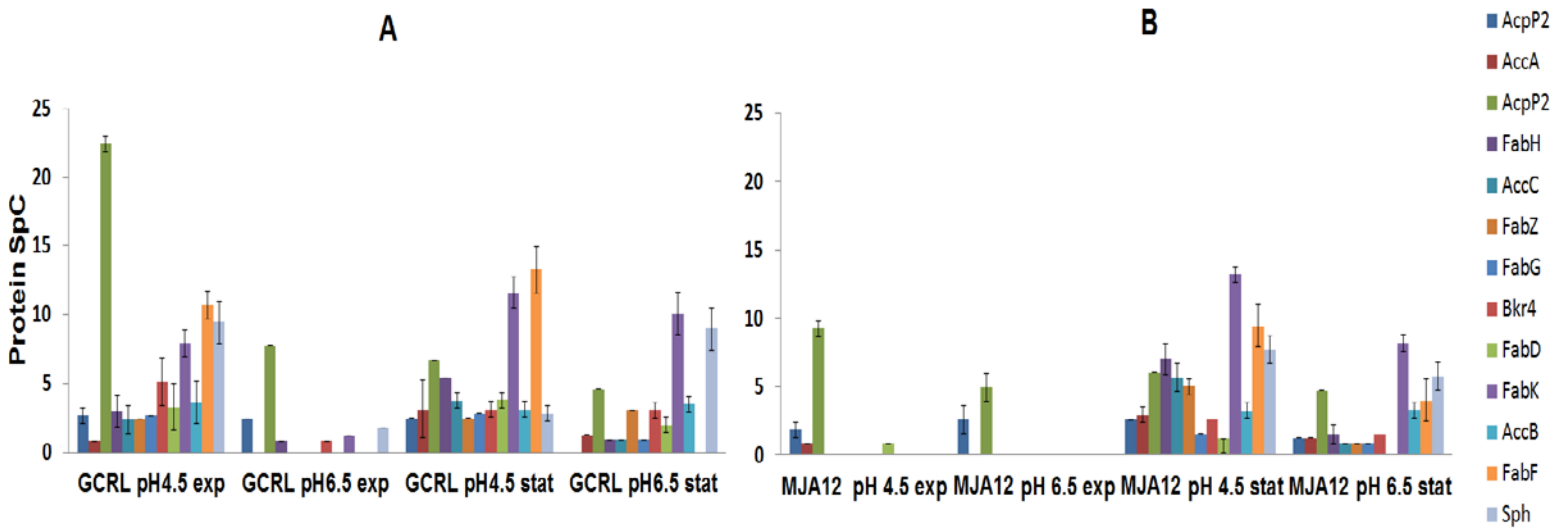


**Figure 4.** Spectral count abundance data for fatty acid metabolism in *Lb. casei* strains A) GCRL163 and B) MJA12 grown anaerobically at different pHs. The SpC values are the average obtained per biological replicate based on spectra that pass filteration criteria. The error bars are the standard deviation values derived from the comparisons. Where values are absent spectra were not observed for the enzyme in the given samples. Abbreviations: exp (exponential growth phase); stat (stationary growth phase). **AcpP2**: acyl carrier protein, **AccA**: acetyl-CoA carboxylase carboxyl transferase subunit alpha, **AcpP2**: acyl carrier protein, **FabH**: 3-oxoacyl-ACP synthase III, **AccC**: acetyl-CoA carboxylase biotin carboxylase subunit, **FabZ**: (3R)-hydroxymyristoyl-ACP dehydratase, **FabG**: 3-oxoacyl-ACP reductase, **Bkr4**: 3-oxoacyl-[acyl-carrier-protein] reductase 4, **FabD**: malonyl CoA-acyl carrier protein transacylase, FabK: enoyl-[acyl-carrier protein] reductase II, **AccB**: acetyl-CoA carboxylase biotin carboxyl carrier protein, **FabF**: 3-oxoacyl-ACP synthase I/II, **Sph**: fatty acid hydratase/isomerase (oleate hydratase, linoleate isomerase).

**Table 1: Proteins that show enhanced abundance in *Lb. casei* strains GCRL163 and MJA12 during exponential growth in MRS at pH 4.5 under anaerobic conditions.**

| Protein identified | | Functional Group | GCRL163 | *P* | MJA12 | *p* |
|---|---|---|---|---|---|---|
| | | | **Fold-change** | | **Fold-change** | |
| dTDP-4-dehydrorhamnose 3,5-epimerase | | Cell wall biogenesis | **35.5** | **0.043** | | |
| D-alanine-poly(phosphoribitol) ligase subunit 2 | DltC | Cell wall biogenesis | **4.1** | **0.007** | | |
| ATP synthase subunit delta | AtpH | Membrane bioenergetics | **4.0** | **0.002** | 1.6 | 0.399 |
| ferredoxin--NADP reductase | YumC | Membrane bioenergetics | 1.3 | 0.365 | 4.2 | 0.067 |
| bifunctional transcriptional regulator (WHTH_GntR superfamily) fused with a PLP dep. ARO8-like aminotransferase family protein | | Amino acid-related metabolism | **34.3** | **0.000** | | |
| S-ribosylhomocysteine lyase | LuxS | Amino acid-related metabolism | **9.5** | **0.020** | 0.8 | 0.309 |
| oligoendopeptidase F | YjbG2 | Amino acid-related metabolism | **6.6** | **0.006** | | |
| cysteine synthase | CysK | Amino acid-related metabolism | **6.2** | **0.000** | 1.2 | 0.691 |
| pyrroline-5-carboxylate reductase | ProC | Amino acid-related metabolism | **5.3** | **0.025** | 0.4 | 0.374 |
| proline iminopeptidase | Pip2 | Amino acid-related metabolism | **5.3** | **0.025** | **4.2** | **0.007** |
| tripeptide aminopeptidase T | PepT | Amino acid-related metabolism | 4.7 | 0.115 | **19.1** | **0.001** |
| oligoendopeptidase F | YjbG | Amino acid-related metabolism | 4.7 | 0.057 | **2.3** | **0.028** |
| glutamyl aminopeptidase, | YsdC | Amino acid-related | 1.0 | | **7.5** | **0.001** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| M20_dimer superfamily | | metabolism | | | | | |
| branched-chain amino acid aminotransferase | IlvE | Amino acid-related metabolism | **2.5** | **0.019** | **5.7** | **0.001** |
| Xaa-Pro dipeptidyl-peptidase | PepX | Amino acid-related metabolism | **2.9** | **0.000** | **4.4** | **0.035** |
| Phosphoribosylaminoimidazole-succinocarboxamide synthase | PurC | Nucleic acid/nucleotide metabolism | **25.1** | **0.000** | | |
| phosphoribosylaminoimidazole carboxamide formyltransferase/inosine-monophosphate cyclohydrolase | PurH | Nucleic acid/nucleotide metabolism | **17.1** | **0.000** | | |
| aspartate carbamoyltransferase | PyrB | Nucleic acid/nucleotide metabolism | **13.3** | **0.000** | 2.3 | 0.374 |
| carbamoyl-phosphate synthase large subunit | CarB | Nucleic acid/nucleotide metabolism | **7.9** | **0.000** | 0.7 | 0.486 |
| N5-carboxyaminoimidazole ribonucleotide mutase | PurE | Nucleic acid/nucleotide metabolism | 7.2 | 0.066 | | |
| N5-carboxyaminoimidazole ribonucleotide synthase | PurK2 | Nucleic acid/nucleotide metabolism | 5.9 | 0.091 | | |
| phosphoribosylformylglycinamidine synthase | PurS | Nucleic acid/nucleotide metabolism | **5.9** | **0.001** | | |
| anaerobic ribonucleoside-triphosphate reductase | NrdD | Nucleic acid/nucleotide metabolism | 5.3 | 0.124 | 0.4 | 0.116 |
| dihydroorotase | PyrC | Nucleic acid/nucleotide metabolism | **4.7** | **0.026** | 0.6 | 0.374 |
| 3-oxoacyl-ACP synthase I/II | FabF | Lipid-related metabolism | **21.4** | **0.000** | | |
| acetyl-CoA carboxylase biotin carboxyl carrier protein | AccB | Lipid-related metabolism | **7.2** | **0.020** | | |
| enoyl-[acyl-carrier protein] reductase II | FabK | Lipid-related metabolism | **6.9** | **0.000** | | |
| malonyl CoA-acyl carrier protein transacylase | FabD | Lipid-related metabolism | **6.6** | **0.040** | 1.6 | 0.374 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3-oxoacyl-[acyl-carrier-protein] reductase 4 | Bkr4 | Lipid-related metabolism | **6.4** | **0.011** | | |
| 3-oxoacyl-ACP reductase | FabG | Lipid-related metabolism | **5.3** | **0.002** | | |
| fatty acid hydratase/isomerase (oleate hydratase, linoleate isomerase) | Sph | Lipid-related metabolism | **5.3** | **0.002** | | |
| (3R)-hydroxymyristoyl-ACP dehydratase | FabZ | Lipid-related metabolism | **4.7** | **0.000** | | |
| acetyl-CoA carboxylase biotin carboxylase subunit | AccC | Lipid-related metabolism | **4.7** | **0.026** | | |
| mevalonate kinase | MvaK1 | Lipid-related metabolism | 0.9 | 0.733 | **4.4** | **0.017** |
| ferrochelatase | HemH | Cofactor-related metabolism | **4.7** | **0.026** | 0.4 | 0.116 |
| riboflavin biosynthesisn acetyltransferase | RibT | Cofactor-related metabolism | **4.7** | **0.025** | 0.6 | 0.374 |
| ATP-dependent helicase/nuclease subunit A | AddA | DNA repair/recombination | **5.9** | **0.016** | 0.6 | 0.374 |
| ATP-dependent chaperone/Clp protease | ClpB | Protein folding/turnover | 1.9 | 0.115 | **8.1** | **0.005** |
| ATP-dependent Clp protease ATP-binding subunit ClpE | ClpE | Protein folding/turnover | **2.5** | **0.017** | **4.1** | **0.004** |
| glutathione peroxidase | Gpo | Cell defense/detoxification | **7.2** | **0.007** | 1.7 | 0.139 |
| esterase/lipase, Aes superfamily protein | | General prediction only | **9.6** | **0.000** | 1.6 | 0.374 |
| NADPH-dependent FMN reductase with a PAS domain | | General prediction only | 5.9 | 0.091 | 0.5 | 0.357 |
| phosphatidylethanolamine-binding protein, putative | | General prediction only | **5.3** | **0.002** | _[a] | |
| HAD-like hydrolase superfamily protein Gph-like | | General prediction only | **4.5** | **0.000** | 0.9 | 0.611 |
| D-hydantoinase/oxoprolinase, hydantoinase superfamily | HyuA | General prediction only | 0.6 | 0.374 | **31.4** | **0.000** |
| MviM family oxidoreductase | | General prediction only | 1.5 | 0.337 | **6.2** | **0.001** |

| | | | | | | |
|---|---|---|---|---|---|---|
| RNA-binding transcriptional accessory helicase, Tex family | | General prediction only | 0.8 | 0.350 | **4.9** | **0.000** |
| DUF2316 superfamily protein | | Unknown/uncharacterized | 5.9 | 0.140 | 0.6 | 0.374 |
| hypothetical protein BN194_07550 | | Unknown/uncharacterized | **5.6** | **0.013** | 1.0 | 0.952 |
| hypothetical protein BN194_06580 | | Unknown/uncharacterized | **4.7** | **0.000** | | |
| hypothetical protein BN194_01830 | | Unknown/uncharacterized | **4.6** | **0.001** | 1.2 | 0.452 |
| hypothetical protein BN194_20190 | | Unknown/uncharacterized | **4.4** | **0.003** | 2.9 | 0.158 |
| GatB_YqeY superfamily protein | | Unknown/uncharacterized | **4.4** | **0.003** | 1.8 | 0.127 |
| DUF2969 superfamily protein | | Unknown/uncharacterized | **4.1** | **0.016** | 0.8 | 0.556 |
| DUF901 superfamily protein with PIN domain | YacP | Unknown/uncharacterized | | | **10.0** | **0.006** |
| DUF969 membrane superfamily protein | | Unknown/uncharacterized | | | **5.5** | **0.002** |
| hypothetical protein BN194_02480 | | Unknown/uncharacterized | 0.6 | 0.105 | **5.5** | **0.002** |
| oligopeptide ABC-type transporter, ATP-binding protein | OppF2 | ABC-type transporter systems | **7.2** | **0.020** | | |
| ABC-type transporter, ATP-binding protein | YvrO | ABC-type transporter systems | **5.0** | **0.047** | 1.1 | 0.528 |
| di-/oligopeptide ABC transporter substrate-binding protein | DppE/OppA | ABC-type transporter systems | 0.6 | 0.374 | **7.9** | **0.001** |
| di-/oligopeptide ABC transporter substrate-binding protein | DppE/OppA | ABC-type transporter systems | 1.0 | 0.961 | **7.9** | **0.017** |
| oligopeptide uptake ABC-type transporter, substrate-binding protein | OppA3 | ABC-type transporter systems | 0.4 | 0.116 | **7.3** | **0.000** |
| oligopeptide uptake ABC-type transporter, ATP-binding protein | OppF3 | ABC-type transporter systems | 0.6 | 0.374 | **4.9** | **0.026** |
| branched-chain amino acid ABC-type transporter, substrate binding protein | LivK | ABC-type transporter systems | 2.9 | 0.158 | **4.0** | **0.009** |
| pyruvate oxidase | YdaP | Carbohydrate-related | 1.2 | 0.662 | **15.9** | **0.003** |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | metabolism | | | | |
| glycerol dehydrogenase, GlyDH_like1 family, putative | YbdH | Carbohydrate-related metabolism | 0.6 | 0.374 | **7.5** | **0.001** |
| enolase | Eno | Central glycolytic/intermediary pathways | **20.4** | **0.000** | **2.4** | **0.003** |
| dihydrolipoyl dehydrogenase | PdhD | Central glycolytic/intermediary pathways | **4.5** | **0.003** | 0.8 | 0.251 |
| ribulose-phosphate 3-epimerase | Rpe | Central glycolytic/intermediary pathways | 4.1 | 0.132 | 1.6 | 0.374 |
| malate dehydrogenase (malic enzyme) | MleA | Tricarboxylic acid pathway | **0.1** | **0.000** | **10.7** | **0.000** |
| pyruvate carboxylase | Pyc | Tricarboxylic acid pathway | 1.0 | 0.981 | **4.2** | **0.008** |

[a]A blank cell indicates the protein was not detected in the protein extract.

**Several oligopeptide transporters, peptidases and aminotrasferases are boosted in abundance as a means to supply exogenous amino acids during acid stress.** Metabolism or accumulation of amino acids, in particular Asp and His, has been found to be a factor in enhanced acid tolerance in *Lb. casei*. Asp was considered to elevate the cytoplasmic levels of Arg, Leu, Ile, and Val, all of which are essential for growth and could directly or indirectly act to increase $pH_i$ and maintain intracellular ATP levels (Wu *et al*., 2012). Enzymes associated with aspartate metabolism, especially aminotransferases leading to formation of other amino acids, were not elevated directly under acid stress conditions during active growth but were elevated in stationary growth phase cells (Figure C1, Figure 7). Detected enzymes of the histidine biosynthetic pathway and the enzymes converting Asp to Arg (ArgG, ArgH) were at negligible levels thus it is assumed *de novo* synthesis is not used as a major adaptation to acid conditions. It was surmised that acid stress may increase the levels of proteins that are engaged in amino acid acquisition, including transporters and peptidases. Several peptidases show significantly greater abundance when either GCRL163 or strain MJA12 were growing actively at pH 4.5 (Figure 5) including proline iminopeptidase (Pip), tripeptide aminopeptidase T (PepT), oligoendopeptidase F (YjbG), neutral endopeptidase O (PepO), dipeptidase B (PepD), aminopeptidase N (PepN), and bleomycin hydrolase-type aminopeptidase (PepC). These enzymes collectively cleave one to three peptides from the end of protein chains or break down oligopeptides thus supplying amino acids to the cell. Of 31 transporter proteins that were significantly more abundant under either acidic conditions or due to growth phase transition the most pronounced increases in abundance were observed for ABC-type oligopeptide transporters, especially in strain MJA12 (Figure 6). Additional transporter proteins that putatively import polar amino acids (such as L-glutamine and L-histidine), branched chain amino acids, polyamines and calcium ions also increased in abundance, though the responses were strain dependent and most clearly

observed in the biomass collected during stationary growth phase. Branched chain aminotransferase IlvE had increased abundance in biomass collected during active growth at pH 4.5 and during stationary growth phase (Appendix C1). This enzyme generates keto-acids from Ile, Leu and Val, which are potentially converted to their corresponding hydroxyl acidic intermediates via a putative L-hydroxyisocaproate dehydrogenase, which exhibits enhanced abundance in stationary growth phase cells (Appendix C, Figure 7). This activity could serve to contribute $NADH/H^+$ and $NH_4^+$ in the cytoplasm and is known to be associated with flavour development in foods (Ardo *et al*., 2006).

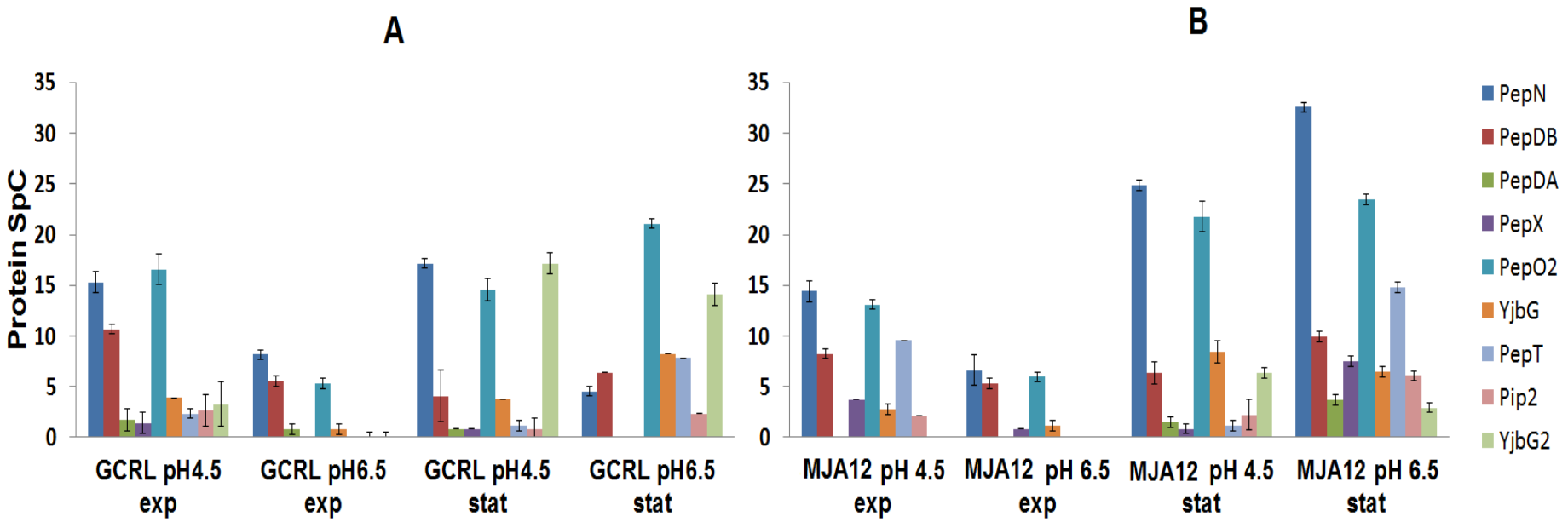


**Figure 5.** Spectral count abundance data for peptidases in *Lb. casei* strains A) GCRL163 and B) MJA12 grown anaerobically at different pHs. See Figure 4 for other detail and abbreviations. **PepN**: aminopeptidase N, **PepDB**: dipeptidase B, **PepDA**: dipeptidase A, **PepX**: dipeptidase A, **PepO2**: neutral endopeptidase, **YjbG**: oligoendopeptidase F, **PepT**: tripeptide aminopeptidase T, **Pip2**: Proline iminopeptidase, **YjbG2**: oligoendopeptidase. F.

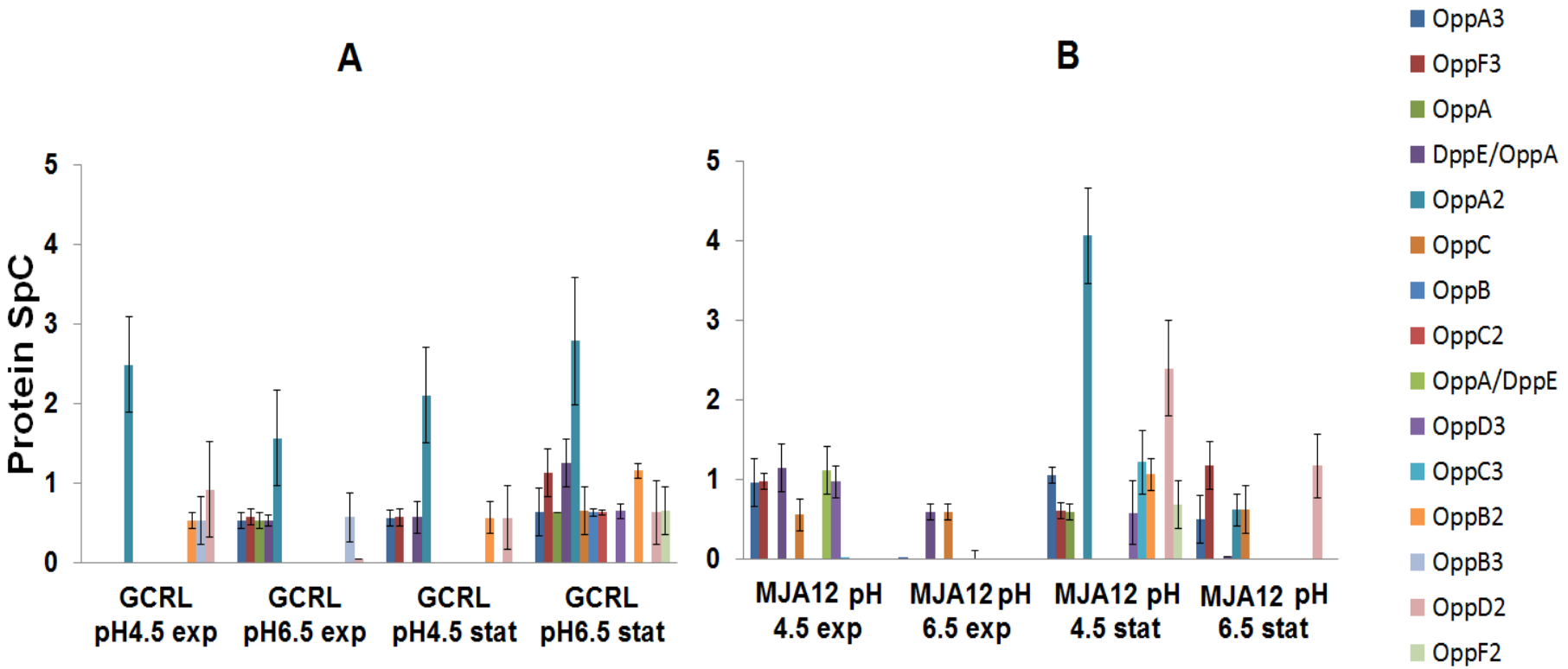


**Figure 6.** Spectral count abundance data for oligopeptide uptake ABC-type transporter proteins in *Lb. casei* strains A) GCRL163 and B) MJA12 grown anaerobically at different pH. See Figure 4 for other details and abbreviations. **OppA, OppA2, OppA3, DppE/OppA**: di-/oligopeptide uptake ABC-type transporter substrate-binding protein; **OppB, OppB2, OppB3**: oligopeptide transport system permease, **OppC, OppC2, OppC3**: oligopeptide transport system, permease, **OppD2**, **OppD3, OppF2**, **OppF3**: oligopeptide uptake ABC-type transporter, ATP-binding protein.

**Contribution of energy yielding pathways during acid stress and stationary growth phase.** Acid stress may exhaust the cellular ATP pool due to increased ATPase $H^+$ extrusion activity. Enhanced activity thus would lead to an expected compensatory increase in ATPase subunit abundance. An assessment of the abundance of the $F_1F_0$-ATPase complex leads to the conclusion that strain GCRL163 does not employ the ATPase complex to drive $H^+$ extrusion to a substantial degree, (Figure 7). Strain MJA12 only exhibits an increased abundance of most of its ATPase subunits in the stationary growth phase when under acid stress, suggesting $H^+$ extrusion could be more important at this growth phase, but otherwise behaves similarly to GCRL163. Since fermentation supplies ATP and NAD(P)H+$H^+$ to the cell, increased abundance of associated enzymes might be expected to aid in energy homeostasis during acid stress. The abundance of enzymes associated with catabolism of sugars and other fermentable substrates was generally not directly affected by acidic pH, but abundance seems instead tied to the growth phase status of the cell population. Though phosphoglycerate kinase (Pgk) levels were unchanged, enolase (Eno) levels were observed to be substantially boosted in abundance during active growth at pH 4.5 for both strains (2.5-20-fold). This response potentially represents a compensation for reduced cellular levels of the phosphodonor phosphoenolpyruvate, which is needed to activate sugars for uptake and fermentation. The most abundant catabolic enzymes, associated with the metabolism of D-galactose, D-glucose, and D-fructose, exhibited slightly greater abundance in stationary growth phase cultures. The abundance of L-lactate and D-lactate dehydrogenase, the major enzymes present leading to end-product formation (and resupplying $NAD^+$), was relatively unchanged in GCRL163. The responses of citrate and malolactic fermentation associated enzymes, the latter consuming $H^+$ in the conversion of malate to lactate, also showed growth phase dependency in GCRL163 with citrate lyase being more abundant in stationary growth phase while malic enzyme (oxaloacetate decarboxylating, EC 1.1.1.38), was most abundant at

pH 6.5. Strain MJA12 showed a different response with malic enzyme much more abundant during active growth at pH 4.5 than GCRL163 while citrate lyase abundance was low under all conditions (Figure Appendix C3, Figure 7). The data suggests that *Lb. casei* strains employ a largely similar strategy to gain energy under acidic stress though malolactic fermentation as a means to limit $H^+$ and generate PEP in the cytoplasm seems strain-dependent.

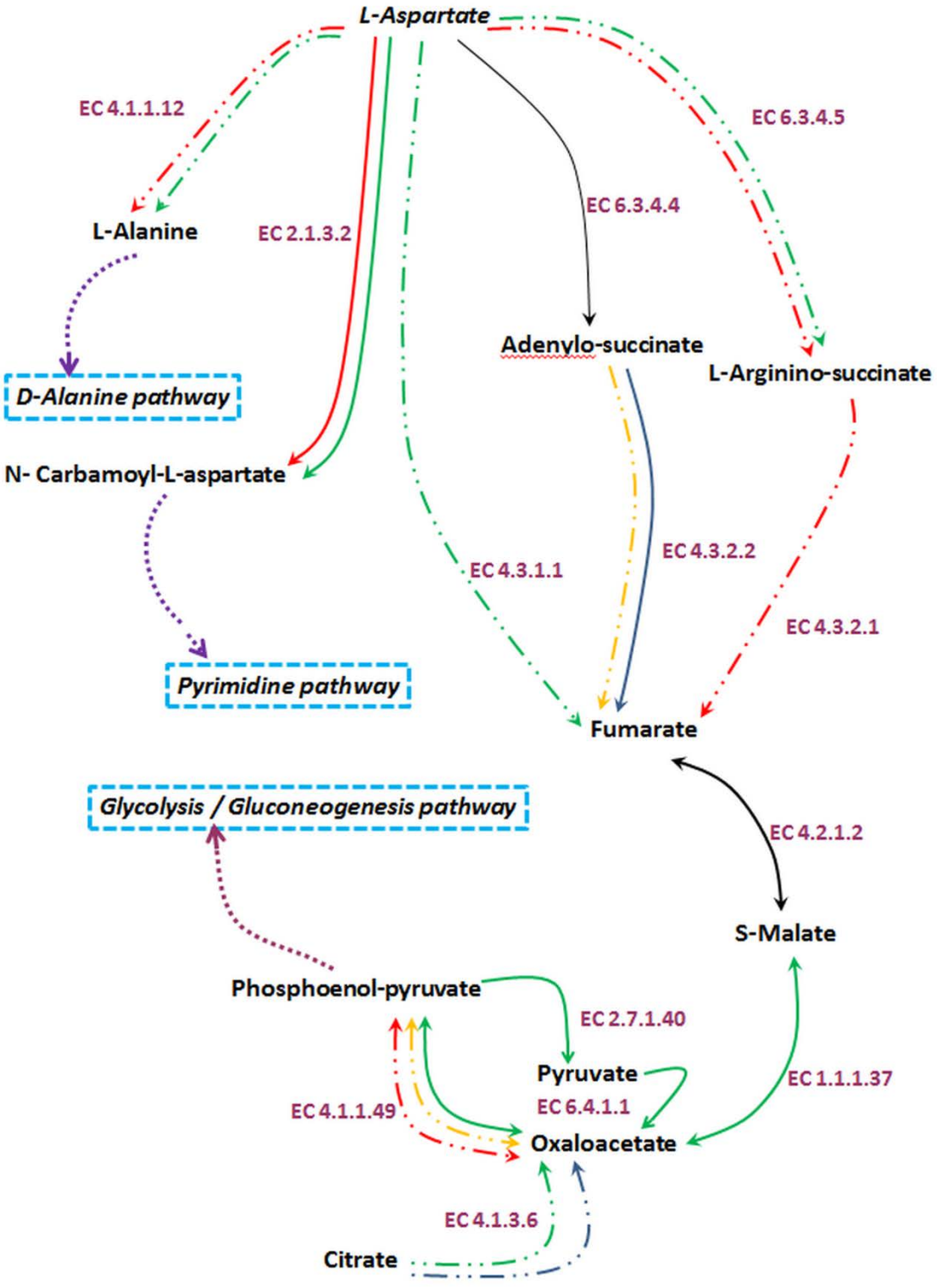
L-Aspartate
EC 4.1.1.12
EC 6.3.4.5
EC 6.3.4.4
L-Alanine
EC 2.1.3.2
Adenylo-succinate
L-Arginino-succinate
D-Alanine pathway
N- Carbamoyl-L-aspartate
EC 4.3.2.2
EC 4.3.1.1
EC 4.3.2.1
Pyrimidine pathway
Fumarate
Glycolysis / Gluconeogenesis pathway
EC 4.2.1.2
S-Malate
Phosphoenol-pyruvate
EC 2.7.1.40
Pyruvate
EC 1.1.1.37
EC 4.1.1.49
EC 6.4.1.1
Oxaloacetate
EC 4.1.3.6
Citrate

**Figure 7.** Metabolic map of intermediary metabolism of *Lb. casei* GCRL163 and MJA12 when grown at low pH 4.5 relative to the control at pH 6.5. The map was constructed from spectral abundance data and demonstrates relative abundance of pathway components during growth at low pH. Pathway assignments of proteins are based on information from the KEGG database. Enzyme designations are given as their corresponding E.C. code. The type of the line is indicative of the protein abundant, sold line indicates proteins significantly more abundant than the overall mean protein SpC level and dashed lines indicate enzymes of low abundance. Red and yellow lines indicate strain GCRL163 at exponential and stationary growth phases, respectively. Green and blue lines indicate strain MJA12 at exponential or stationary growth phases, respectively. Lines that are black in color indicate proteins that were essentially undetectable with the approach used.

**Acid stress enhances the nucleic acid and nucleotide biosynthetic pathway in *Lactobacillus casei*.** Acid stress increased most nucleic acid biosynthetic and several downstream nucleoside biosynthetic and salvage pathway enzymes, with this trend becoming more obvious in stationary growth phase. Given acidity and stationary phase are slow growth rate states and the demand for new DNA would be lower, this response seems to be compensatory in nature, possibly due to disruption of availability of certain precursors to the pathways, such as glutamine, or reduced functionality of the enzymes when the $pH_i$ becomes increasingly acidic. The latter is suggested by an increase in abundance (seven to eight fold) of RecA in stationary growth phase accompanied by increased abundance of AddA and AddB, involved in repairing double-strand breaks as well as two PcrA-like DNA helicases that are putatively involved in DNA repair.

**Acidification of the cytoplasm does not enhance protein turnover enzyme abundance but is associated with reduced nascent protein folding enzyme levels.** The slow growth at pH 4.5 is presumed to be due to acidification of the cytoplasm and enervation of the cells as the ATP pool is depleted. The protein data suggests that an outcome of the acidification is reduced abundance of proteins involved with primary folding of nascent proteins (GroEL, GroES, DnaK, DnaJ, GrpE, Tig). Stress or shock responsive alpha-crystalline domain proteins (Hsp18.5 and Hsp19.3) that may have a role in protein disaggregation (Spano *et al*., 2005) were abundant in actively growing cells at pH 6.5 but were reduced in abundance (by >90-fold) in slow growing and stationary growth cells. Despite acidic conditions leading to reduced $pH_i$ there was no evidence of increases in the abundance of enzymes involved in turnover of defective, misfolded proteins, including most of the Clp system enzymes as well as other enzymes such as HtrA (DegP), HslV/HslU, and a Lon protease homolog (YlbL). This could be due to slower protein synthesis and folding rates leading to apparent lack of increased recycling of proteins that might be otherwise more important in situations when sudden acid stress occurs (Wall *et al*., 2007).

**Discussion:**

Acid adaptation has been proposed as a selection criterion for probiotic bacteria (Collado *et al*., 2007). The ability to adapt and survive in acidic environments is an important function of *Lb. casei* as a probiotic agent. Most of the published works that have examined *Lb. casei* in relation to acid stress have done so under scenarios relevant to sudden exposure to gastric juices. This typically involves an exposure at about pH 2.0-2.5 (adjusted with HCl) for two hours (Lee *et al*., 2008; Fernandez *et al*., 2008; Broadbent *et al*., 2010). This study focused on examining acid adaptation instead, and takes into account the combined effects of $H^+$ and organic acids. Relatively high coverage of the proteomes allowed for the first

comparisons of different *Lb. casei* strains (GCRL163 and MJA12) derived from different fermented food products and that on the basis of MLST analysis are non-clonal. The study takes into account the possibilities of genetic as well as growth phase-related effects on acid resistance.

Here we report that in response to adapted growth under low pH, GCRL163 demonstrates different survival strategies compared to MJA12. In relation to broad scale proteome responses and functional trends (Figure 2 and 3), differences between the two strains were observed. GCRL163 appeared to acid adapt during the exponential growth phase while MJA12 seemed to mainly attain full acid adaptation in the stationary growth phase. Acid adaptation has been reported for other LAB (Broadbent *et al*., 2010; Koponen *et al*., 2012). Broadbent *et al*. (2010) demonstrated that *Lb. casei* ATCC334 when exposed to solutions of pH from 3 to 5 for 1 hour and then challenged at pH 2, the highest survival was found for the bacteria adapted at pH 4. The analysis of the membrane fatty acid in acid adapted cells demonstrated a higher total percentage of saturated and cyclopropane fatty acids compared to control, and that a stringent-type response was invoked. Our study supports the findings of Broadbent and colleagues, as proteins involved in fatty acid biosynthesis were observed to become more abundant suggesting enhanced synthesis of new fatty acids and/or more rapid turnover of fatty acids in the cell membrane. Fozo *et al*. (2004) and Broadbent *et al*.,(2010) have previously reported that cultures grown at lower pH increased proportions of oleic acid and cyclopropane nonadecylic acid, with concomitant decreases in hexadecanoic acid and cyclopropane heptadecanoic acid.

It has been suggested that the fatty acid composition of the membrane can influence proton permeability. This indicates that membrane composition plays an important role in the

defense against acid stress (Wu *et al*., 2012). This strategy is also used by other *Lactobacillus* strains to withstand various environmental stresses. For example, Zhai *et al*. (2013) demonstrated that *Lb. delbrueckii* subsp. *bulgaricus* CAUH1 rerouted pyruvate metabolism to fatty acid biosynthesis, aiding possible modifications of the cell membrane when cultures were grow under low pH. The same mechanism was observed in other Gram-positive bacteria, including *Listeria monocytogenes* and *Streptococcus mutans (*Zhai *et al*., 2013). *Lb. casei* increased the abundance of proteins related to accumulation of amino acids and degradation of oligopeptides. This strategy has been demonstrated to have several physiological roles including intracellular pH control and generation of energy (Fernández *et al*., 2006). Enzymes related to amino acid metabolism, especially aspartate aminotransfer reactions, were mostly increased in abundance at low pH while in stationary growth phase, which indicates that these enzymes play a role in adaptation to suboptimal growth conditions. This response has been observed in other LAB when grown under low pH conditions, such as *Lb. rhamnosus* GG and *Lb. casei* Zhang (Wu *et al*., 2011; Koponen *et al*., 2012) though the details on which proteins are involved were not investigated. Here we can show that a wide range of peptidases appear to be increased in abundance in tandem with a variety of transporters, mostly of the ABC-type, with a focus on oliogopeptides and polar amino acids.

In this study, enzymes related to energy yield show different abundances under the growth conditions examined in by this study. Proteins related to carbohydrate metabolism were more abundant at stationary growth phase in GCRL163 and MJA12 (Appendix C2). The proteomic data suggests that aspects of fermentation metabolism vary between the strains tested. L-lactate and D-lactate dehydrogenase were more abundant at stationary phase for strain MJA12, however these enzymes were not significantly altered in abundance in GCRL163. Enzymes involved in citrate and malolactic fermentation demonstrated strain

dependent changes as well. Malate decarboxylated produces L-lactate and $CO_2$ (Renault *et al*., 1988) and contributes to alkalinization of the cytoplasm and allows ATP generation through $H^+$-ATPase (Poolman *et al*., 1991). Malolactic proteins have been associated in lactic acid bacteria with enhanced survival under acidic conditions (Broadbent *et al*., 2010). In this study, proteomic data of *Lb. casei* MJA12 support the known mechanism of using the malolactic proteins for culture grown under low pH. Citrate fermentation was also strain-phase dependent, and its utilization may also provide ATP for pH homeostasis, and these enzymes key in supporting transporter activity for importing amino acids that are metabolized to neutralize the cytoplasm (Al-Naseri *et al*., 2013). These results are collectively summarised in a metabolic map (Figure 7), showing strain and growth phase differences in key central metabolic enzymes and pathways.

A number of proteins that play a role in nucleic and nucleotide biosynthesis pathways were elevated in response to the growth under low pH. The DNA repair and recombination protein RecA increased in abundance in both strains in stationary growth phase, presumably repairing damaged DNA created by acid stress (Hartke *et al*., 1996). Damage repair seems to be the ultimate mechanism of resistance against acid stress and other stresses (Hartke *et al*., 1996). (Hartke *et al*., 1994; Quivey *et al*., 1995), observed that several proteins related to DNA repair became more abundant in *Lb. lactis* after acid shock. Other studies indicate that induction of the RecA-DNA repair system in acid adapted *Streptococcus mutans* also improved overall resistance to DNA damage (Wickner *et al*., 1999).

One of the mechanisms that bacteria often use when under stress is to increase production of proteins that are involved in folding and turnover, including chaperones like DnaK that rescue proteins and hinder aggregation (Champomier-Verges *et al*., 2002; Hartl *et*

*al*., 2002). Clp ATPase and ClpP proteolytic complexes play indispensable roles in cellular protein quality control systems by refolding or degrading damaged proteins in both stressed and non-stressed cells. A proteomic review study by Champomier-Verges *et al*. (2002) on several acid shock adapted LAB revealed that chaperones were usually up-regulated presumably to repair acid induced damaged proteins or to facilitate the folding of newly synthesized proteins. However, the subset of acid induced heat shock proteins varies between species, with DnaK and GroEL often identified (Champomier-Verges *et al*., 2002; Lorca *et al*., 2002) likely due to their high abundance. In *Lb. acidophilus*, the proteins involved with protein folding such as DnaK, DnaJ, GrpE, GroES, and GroEL are produced as a response to acid adaptation (Wall *et al*., 2007). Further, the level of GroES, GroEL and DnaK increased in an acid-sensitive *Lb. casei* Zhang grown at low pH compared to wild type cells grown at the same pH. However, a study by Wall *et al*. (2007) on the acid stress response of *Lb. reuteri* showed that heat shock proteins did not significantly respond under acidic conditions. Here protein folding protein abundance was reduced in GCRL163 and MJA12 adapted to growth at pH 4.5 compared to pH 6.5. Hence, we hypothesize that more slowly growing strains simply have reduced rates of protein synthesis and turnover. Thus the difference between acid shock and post-adaptation experiments must be considered in the light that the adaptation process is akin to a lag phase whereby the cell must commit metabolic energy to obtain a physiological state optimal to the prevailing environmental conditions. In this sense existing studies primarily have looked at the early stages of acid adaptation where in this study the later and end stages of acid adaptation is shown.

In conclusion, we describe the important features of *Lb. casei* strains achieve once acid adapted. This is relevant in understanding how *Lb. casei* isolates subsist and survive in fermented food. The data suggested that the two *Lb. casei* studies show responses that are

growth phase, pH, and strain dependent, with the first being most influential overall. Intracellular accumulation of amino acids seems quite active and thus an exogenous supply of protein may aid long-term survival of *Lb. casei* in acidic foods. This and the adroitness of cell membrane fatty acid modulation is likely additive in effecting the rates at which $H^+$ and organic acids accumulate in the cells. The differences in the proteomes between the strains, as suggested at the metabolomic level responses, deserve attention in understanding how probiotics can be better applied in food systems and how adaptation responses may alter product traits when non-starter LAB survive for long periods in food products.

# CHAPTER FIVE

# Proteomic Analysis of Lithium Chloride Extracts of *Lactobacillus casei*: Evaluation of Impact of Growth at Low pH on Surface Protein Composition and Bacterial Adhesion to HT-29 Cells

**Abstract**

The effect of prior growth at low pH of two strains of *Lactobacillus casei* (strains GCRL163 and MJA12) on adhesion was examined using the HT-29 cell line as an *in vitro* model for intestinal epithelium cells. The strains were grown under anaerobic conditions in MRS broth adjusted and maintained at pH 4.5 or pH 6.5 in fermenters, with biomass collected in early-stationary growth phase. *Lb. casei* showed increased attachment to the cell line after adaptation to pH 4.5 compared with cultures grown at pH 6.5 and prior exposure of HT-29 cells to dialysed LiCl extracts inhibited subsequent binding of *Lb. casei* cells. Gel-free proteomic analysis of proteins extracted from whole cells using 5 M LiCl was undertaken to understand the nature of the changes in cell-surface proteins that could be related to adhesion. Relative to the distribution of proteins in cell-free lysates, 5 M LiCl extracts were enriched with mainly of transmembrane and membrane-associated proteins. Cell wall proteins were in general only weakly enriched, with the exception of a putative cell wall-associated hydrolase, while cytosolic proteins were moderately depleted relative to whole cell lysates. Although LiCl extraction was clearly not a sufficiently specific approach to fractionate cell-surface proteins, the method was able to demonstrate changes in abundance of proteins relative to their abundance in cell lysates and changes in abundance following growth at different pHs. The results suggest an alternative fractionation approach to study cell surface proteins in *Lactobacillus* is required to differentiate sub-cellular location of several of the moonlighting proteins detected in LiCl extracts.

## Introduction

Probiotic microorganisms, when consumed in adequate amounts, potentially confer a health effect on the host (Joint *et al*., 2006). Typically probiotic bacteria include members of the genera *Lactobacillus* and *Bifidobacterium*. These bacteria are Gram-positive, non-pathogenic, non-sporulating aerotolerant anaerobes that form lactic acid as the main end-product of carbohydrate fermentation. Probiotic benefits conferred by lactobacilli and bifidobacteria colonizing the gastrointestinal tract can potentially include modulation of the immune system, lowering serum cholesterol levels, alleviating or preventing intestinal disorders and inhibiting pathogen adhesion (Taverniti *et al*., 2013, Woo and Ahn, 2013). With the exception of antimicrobial activity and organic acid production, molecular mechanisms underlying such effects on the host still remain unclear.

Fermented products containing *Lactobacillus* spp. are regarded as safe (Saxelin *et al*., 1996) and are used as a major means to deliver probiotic strains (Puertollano *et al*., 2008). Strains of several species also play a role in the manufacture of fermented foods, where they influence traits such as flavour development and improve shelf-life, thus combining fermented food production with the inclusion of proven probiotic strains is a means of improving health benefits of these foods (Awad *et al*., 2007; Saxelin *et al*., 1996). To impart probiotic functionality, strains must be able to survive and compete during product manufacture and storage, and subsequently survive passage through, plus retention in, the gastrointestinal tract.

Adhesion and possible penetration through mucus layers are important probiotic characteristics that may contribute to transient colonization of the gastrointestinal tract and competitive exclusion of pathogens. The capacity to adhere to epithelial cell layers may also promote direct interaction with immune system cells of the lamina propria (Sanchez *et al*.,

2008). The ability of probiotic strains to adhere to epithelial cells of the gastrointestinal tract is commonly tested *in vitro* using different immortalised epithelium cell lines (Altermann *et al*., 2005), such as HT-29 (Sulaiman *et al*., 2009) and Caco-2 (Tuomola and Salminen, 1998), or mucus (Ouwehand *et al*., 1999). Adhesion to HT-29 cells is mediated by several factors on the cell surface of Gram-positive microorganisms, including lipoteichoic acid, mucus- and fibronectin-binding proteins (Selle and Klaenhammer, 2013). More specifically, the adhesion ability of *Lactobacillus* is linked to cell surface proteins, where the first interaction happens between the bacteria and the host cell surfaces (Deepika and Charalampopoulos, 2010). There are several factors that likely influence the surface properties of lactobacilli. It has been suggested that these properties are influenced by the physiological state of the cells, and are also associated with changes in the fatty acid composition, the membrane permeability, and enzymatic activities of the cells (Wang *et al*., 2005; Carvalho *et al*., 2004). The fermentation medium, incubation conditions, incubation time, and post-fermentation processing (including harvesting, freezing, freeze drying, storage and the food matrix) could potentially affect these cellular properties (Wang *et al*., 2005). The rationale behind this assertion is that the fermentation and downstream processes are likely to result in compositional, organizational, and conformational changes at the bacterial surface, which are consequently likely to affect surface electrostatic properties and adhesion abilities (Deepika and Charalampopoulos, 2010). Further, cell surface proteins may protect the bacterial cell from various environmental factors such as mechanical and osmotic stresses (Engelhardt, 2007a; Engelhardt, 2007b). In the genus *Lactobacillus*, the presence of a monomolecular layer composed of the S-layer family proteins (SLAP) has been associated with adhesion and protective properties (Glenting *et al*., 2013). However S-layer proteins are poorly conserved and do not occur in all species. *Lb. kefir*, *Lb. helveticus* and *Lb. hilgardii* and organisms of the former *Lb. acidophilus* group carry predicted S-layer protein genes in their sequenced genomes (Garrote

*et al*., 2004). No S-layer proteins have been observed on the surface of *Lb. casei*, *Lb. paracasei* subspecies *paracasei* and *Lb. rhamnosus* nor do SLAP genes occur in genomes of these species (Boot *et al*., 1996; Zhang *et al*., 2010; Hynonen and Palva 2013).

In recent decades, information about the biological functions of cell surface proteins has accumulated in many Gram positive species. The primary functions characterized thus far include acting as binding sites for large molecules (Peters *et al*., 1995) and as mediators of bacterial adhesion (Poppinga *et al*., 2012). Nezhad *et al*., (2012) showed that putative cell-surface-associated proteins were extracted from *Lb. casei* strain GRCL46 using LiCl, and that the composition of the extracts was influenced by growth at low pH. Many of the proteins detected were "moonlighting proteins", proteins with known functions in the cytoplasm (such as central metabolism enzymes) but have been suggested to have different functions when located at the cell surface (Jeffery *et al*., 1999; Huberts and Klei, 2011), where some are associated with glycolysis – notably glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and enolase. Moonlighting proteins are associated with the bacterial surface by electrostatic linking to cell wall components or to the cytoplasmic membrane. Limited evidence exists on how these proteins have been mobilized to the surface but it is assumed to be passive or may involve adhesion to bacterial cells by cytosolic proteins released during growth due to lysis (Kainulainen and Korhonen 2014). The secondary functions have only been experimentally confirmed in some cases (Huberts and Klei 2011). Nezhad *et al*., (2012) noted that the proteins extracted from *Lb. casei* were present at low density, needing to be concentrated to demonstrate their presence. Similar observations were made by (Hurmalainen *et al*., 2007), who demonstrated that proteins of several lactobacilli species leached into extracellular environment at pH >7 over time but that the relative amount of proteins detected for the *Lb. casei* group was low. Whether these proteins are contaminants from the cytoplasm or indeed

have multitasking roles is a topic under considerable study at present (Copley 2012; Henderson and Martin 2011; Sirover 2011; Wang *et al*., 2013).

To understand further how growth conditions may influence the composition of the cell surface proteome of *Lb. casei*, LiCl extraction and proteomic analysis were employed in the present study to investigate strains GCRL163 (cheese isolate) and MJA12 (from a fermented milk drink containing a probiotic) grown anaerobically at different pHs. The goals of the research were to determine i) the effect of medium pH on the adhesion properties of *Lb. casei*; ii) the efficiency of LiCl extraction in enriching surface proteins from cells grown at different pH; and iii) whether growth at low pH leads to enrichment of cell surface proteins that might be linked to adhesion mechanisms.

## Material and methods

### Bacterial strain and growth conditions

Two *Lactobacillus casei* strains GCRL163, a Cheddar cheese isolate (Chandry *et al*., 2002), and MJA12, derived from the Yakult milk product (Yakult Australia) were used. Cells were passaged through two sequential subcultures in MRS broth incubated at 37 °C for 12 h under anaerobic conditions (Anaerocult A system, Oxoid, Australia). Appropriate dilutions of cultures grown for 12 h in MRS broth were plated onto MRS agar and incubated anaerobically at 37°C for 48 h. A single colony from the plate of each strain was resuspended in MRS broth. One individual colony represented one biological replicate, and this step was conducted three separate times. Bacterial cells were harvested at early stationary growth phase and inoculated at 2% (v/v) into 900 ml of MRS in two sets of three Bioflo/Celligen 115 Benchtop Fermentor/Bioreactor systems (New Brunswick, an Eppendorf company, USA).

**Growth at set pH values**

Within bioreactors, cultures were grown at 30°C with agitation at 100 rpm. Two sets of three bioreactors were maintained at pH 4.5 (low pH) and 6.5 (control), cell growth was monitored by measuring OD and determining viable counts on MRS agar plates. The pH was controlled by bioreactors through the additions of 2 M HCl and 2 M NaOH. Prior to inoculation, media was rendered anaerobic by sparging with nitrogen gas with $O_2$ levels monitored with a dissolved $O_2$ probe, with gas addition controlled to maintain strictly anaerobic conditions. Cells from early stationary growth phase were collected from 450 ml of culture broth by centrifugation (5,000 rpm, 10 min, 4 $^{o}$C) and the cell pellets were washed once with Tris-HCl buffer (0.04 M, pH 7.0). Samples were taken after 10 hours (early stationary phase, pH 6.5) and 20 h (early stationary phase, pH 4.5). Bacterial cells were then immediately used for cell surface protein extraction.

**Cell surface proteins extraction using LiCl**

Cell surface proteins of *Lb. casei* GCRL163 and MJA12 were extracted using LiCl according to a method developed by Lortal *et al.* (1992). Cells, after one wash with Tris-HCl buffer (0.04 M, pH 7.0), were re-suspended in 5 M LiCl using 10-15 mg wet weight of cells/mL (Lortal *et al*., 1992). Cell suspensions were incubated with gentle shaking at 4 ºC for 60 min. After centrifugation at 22,000×g for 30 min (Eppendorf 5417C Centrifuge), the supernatant fluids were collected and filtered through 0.2 μm nitrocellulose membranes (Millipore, Australia) then dialyzed (dialysis tubing pore size molecular weight cut-off ≤1,200, retention of compounds with a molecular weight >2,000, Sigma, USA) against 5L of deionized water (4 $^{o}$C), with deionized water changed three times (1, 3 and 6 hours, then left overnight) to remove LiCl. The proteins were then concentrated by laying the dialysis tubes on spectra/gelTM absorbent (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA),

reducing the extract volume from 50 ml to 0.5-1 ml. The concentration of protein in each sample was determined using a BCA kit (Thermo Fisher Scientific, USA) with bovine serum albumin as a standard. Equal amounts of the sample buffer (1:1) were added to the protein samples and mixed by vortexing for 30 s, after which they were heated at 100 ºC for10 min. The samples were then centrifuged to remove any remaining debris. Six µg of protein was loaded in each lane of the SDS-polyacrylamide gels (resolving gel, 10%; stacking gel, 4%) and run at 100 V for 90 min. Following electrophoresis, the gels were visualized by silver staining (Al-Naseri *et a*l., 2013).

**Adhesion assay**

The human colon adenocarcinoma cell line HT-29 (Cellbank Australia) was cultured at 37 °C under an atmosphere of 5% $CO_2$ in Dulbecco's Modified Eagle Medium (DMEM; Merck, Germany) supplemented with 10% (v/v) fetal calf serum, 100 U $ml^{-1}$ penicillin and 100 mg/ $ml^{-1}$ streptomycin. For adhesion assays HT-29 monolayers were prepared on glass cover slips and placed in 6-well tissue culture plates according to the method described previously (Sulaiman *et a*l., 2009). The cells were incubated for four days to reach confluence for use in adhesion assays. The cell culture media was changed every day and replaced by fresh non-supplemented DMEM at least 3 h before the adhesion assays. The adherence of *Lb. casei* strains on HT-29 cell culture was examined using two different methods. The first method involved adding suspensions of either strain GCRL163 or MJA12 (approximately $10^7$ cells) to each of the 6 wells tissue culture plate and incubating at 37 °C in an atmosphere of 5% $CO_2$ for 60 min. Secondly, 10 µg of the cell surface protein extract from cells grown at pH 4.5 was added to each of the 6 wells tissue culture plate and incubated for 60 min followed then by addition of the bacteria cells (grown at pH 4.5) with incubation then performed as described above. After incubation, the HT-29 cell cultures were washed three

times with phosphate-buffered saline (PBS, pH 7.2), fixed with methanol, Gram stained and counted using 20 randomized microscopic fields per cover slip. Each determination was carried out in triplicate (Sulaiman *et al*., 2009).

**LC/MS analysis.**

NanoLC-LTQ-Orbitrap XL tandem mass spectrometry and protein identification were performed as previously described (Al-Naseri *et al*., 2013). The spectral count (SpC), a sampling statistic output of ProteinProphet, was used to determine protein abundance in individual protein samples (Nesvizhskii *et al*., 2003, Liu *et al*., 2004). Differences in the levels of proteins between samples and the significance of these differences were calculated and $Log_2$ fold changes in protein expression under each treatment condition were calculated based on the average SpC, according to the method described by Old *et al*. (2005). Significant differences (*P*-value of <0.05) in protein SpC were normalised and tested for significant differences using the beta-binomial distribution test implemented in R.

**Comparison of LiCl extraction to whole cell extraction**

LiCl extraction SpC data was compared with SpC data obtained from the equivalent stationary growth phase whole cell extracts investigated in Chapter 3. It is assumed the whole cell extracts obtained *via* bead beating contain a wide distribution of proteins from different cell compartments though the predominant proportion will be cytosolic in origin. The exact same cell pellets were used (merely subdivided) to obtain both extract types thus a direct comparison can assess relative extraction biases of proteins from the two different extraction methods. To determine the differences between these extraction methods in obtaining proteins and to determine if the LiCl extraction process is selective, a global analysis of biases in relation to subcellular compartment and secondly on a protein-to-protein basis was

carried out. SpC data derived from both pH 6.5- and pH 4.5-grown samples were pooled (in order to obtain sufficient numbers of comparable proteins) for both strains and the individual strains compared separately. Signal peptide regions and transmembrane helical domains possessed by *Lb. casei* GCRL163 and MJA12 proteins were predicted using SignalP server 4.1 and TMHMM server 2.0 (Emanuelsson *et al*., 2007). Conserved domain structure assignments were used to demarcate proteins to cellular compartments, for example proteins with LPTXG motifs were considered cell wall proteins since this motif indicates the protein is sorted to a peptidoglycan anchor site. Proteins with one THMM domain were designated as membrane-associated since it was assumed they either interact or are anchored in the membrane, either at the inner or outer sides. The resultant subcellular compartments are summarised in Table 1. A single protein may belong to multiple compartments (secreted, membrane and cell wall) while designation of a protein as being secreted only infers it is putatively subjected to intracellular trafficked and is not necessarily mobilised outside of the cell. The proportion of summed SpC within these subcellular compartments and for individual proteins for the different extraction procedures was determined. This was possible as most proteins could be detected in both extracts (after pooling of the pH 4.5 and pH 6.5 culture extracts). To calculate the ratios, contributions of individual proteins to the total SpC pool of each strain were normalised as percentile values (to account for sampling differences); the values were converted to logarithms ($\log_{10}$) and then a correction factor ($\log_{10} = 0.5$) subtracted since the LiCl extract data showed a consistent bias towards enrichment of proteins, which likely derives from the 100-fold concentration needed to allow for LC-MS analysis. The resultant comparison was based on the concept that the abundance of some proteins was the same in each extract type and the enrichment/depeltion ratio should average to zero overall.

## Results

### Growth at low pH enhances *Lb. casei* adhesiveness to HT29 cells.

Adhesion to epithelium cell lines is considered as one of the main criteria for probiotic strains. HT-29 cells has been used as an *in vitro* model for adhesion to screen the adherence capability of bacterial strains (Sulaiman *et al*., 2009, Dhanani and Bagchi, 2013). In this study, we examined the adhesion of *Lb. casei* GCRL163 and MJA12 (Cheese isolate and Yakult fermented milk isolate, respectively) using the HT-29 cell line following growth of the strains at two different pHs. Growth at low pH enhanced the adhesion by 2.25- and 1.71-fold for GCRL163 and MJA12, respectively. MJA12 showed better adhesion properties compared to GCRL163 at both tested pH conditions (Figure 1 A). Adding LiCl extract from cells grown at pH4.5 to HT-29 cells prior to adding *Lb. casei* cells (also grown at pH4.5) significantly decreased the adhesion of both strains (Figure 1 B). This suggests the adhesion properties of both tested strains are at least partly related to proteins extracted by LiCl.

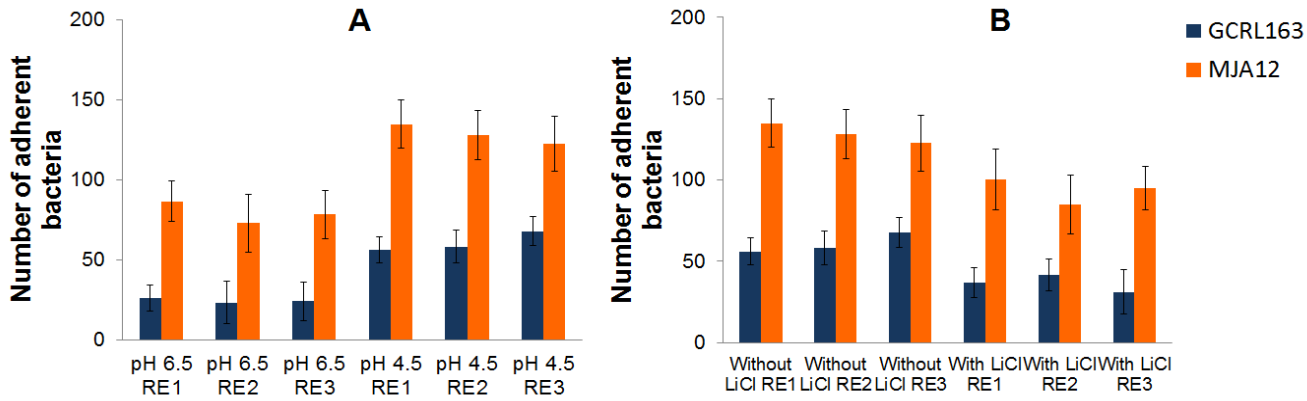


**Figure 1:** Adhesion of *Lb. casei* strains onto monolayers of HT-29 cells. Figures represent the mean and standard deviations of *Lb. casei* strains GCRL163 (Blue) and MJA12 (Orange) adhering per 100 HT-29 cells. Figure (A) shows the adhesion of the strains following growth at pH 4.5 and 6.5, for triplicate replicates (RE1-3); (B) shows adhesion to HT-29 cells which were either treated with 10 µg of protein extract from culture grow at pH 4.5 from the corresponding strain or not.

**SDS-PAGE analysis of LiCl extracts of two *Lb. casei* strains.**

SDS-PAGE analysis of *Lb. casei* GCRL163 and MJA12 grown at two different pHs (6.5 and 4.5) revealed significantly different relative expression of a number of proteins (Figure 2 A, B) at the cell surface. This included changes in the abundance of several proteins bands, especially for bacteria grown in low pH compared to those grown in pH 6.5. The approximate molecular weights of groups of proteins differentially increased in abundance following growth at low pH were ~ 55, ~ 30 kDa in GCRL163 and ~ 40, ~ 30 kDa in MJA12, while several protein bands of ~ 50, ~ 35 kDa in GCRL163 and ~ 70-80, ~ 60 kDa in MJA12 decreased in abundance. The SDS-PAGE data demonstrate that growth at low pH resulted in qualitative changes to the overall proteome of the cell surface in GCRL163 and MJA12. To define the nature of these differences within the proteome, specifically LC/tandem MS-MS analysis was based on in-solution protein samples, allowing discernment of relative abundance changes in detectable proteins.

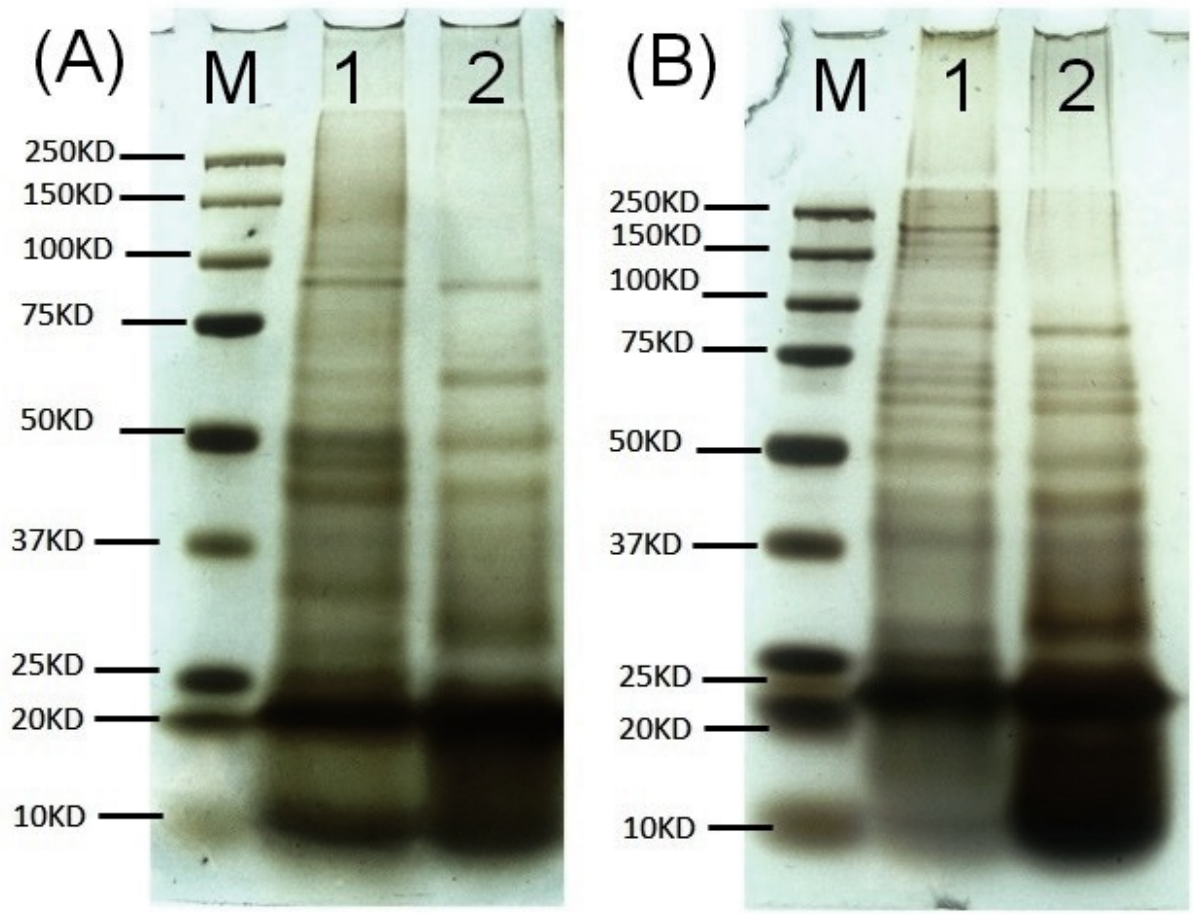


**Figure 2:** (SDS-PAGE of cell surface fractions from *Lb. casei* GCRL163 (A) and MJA12 (B) grown in MRS with fermenters maintained at pH 6.5 or 4.5. Lanes: M, protein ladder; 1 and 2, dialysed and concentrated 5M LiCl extracts of cells after growth at low pH (pH 4.5) control or (pH 6.5), respectively.

**LiCl extracted proteomes of *Lb. casei*.**

In this study, the LiCl extracted proteins of two *Lb. casei,* strains GCRL163 and MJA12, were analysed using nano-LC-LTQ-Orbitrap tandem mass spectrometry and spectral counting f. A total of 380 different proteins were detected that passed stringent filtration criteria. The proteomic analysis revealed several proteins related to different functions: 131 proteins belonged to ABC-type transporter systems, 68 proteins belonged to phosphotransferase systems and 20 putative cell surface proteins (based on criteria in the methods, Table 1) were detected. The majority of the remaining proteins detected were involved in cellular metabolism, with 82 proteins associated with carbohydrate metabolism, 79 with amino acid metabolism, 53 ribosomal proteins and 43 proteins associated with central glycolytic pathways (Figure 3). Sanchez *et al*. (2009) used LiCl to extract cell surface proteins, among the proteins identified in *Lb. rhamnosus* proteins related to ribosomal proteins and other cytosolic proteins similar to what was observed in the present study.

The most abundant proteins that were observed when cultures were grow at pH 6.5 or 4.5 included phosphocarrier protein HPr (9.6% of SpC), cold shock protein (1.7%), GAPDH (1.3%) and elongation factor Tuf (1.2%) (Table 2). The possible presence of these proteins on the cell surface of *Lactobacillus* strains and other bacterial species have been noted previously as “moonlighting proteins” (Deepika *et al*., 2012; Ramiah *et al.*, 2008; Izquierdo *et al*., 2009; Sanchez *et al*., 2009; Bergmann *et al*., 2004; Cathy *et al*., 2013). Furthermore, Nezhad *et al*. (2012) showed that enolase and GAPDH could occur in the extracellular proteome extracts of strain *Lb. casei* GCRL46.

**Table 1: structure assignments used to demarcate proteins to compartments**

| **Compartment** | **Criterion** |
|---|---|
| Cytosolic | Absence of below features |
| Secreted | Signal peptide domain detected. |
| Cell wall | Assigned by domain structure i.e. LPTXG motif |
| Membrane | 2 or more THMM domains |
| Membrane-associated | 1 THMM domain |

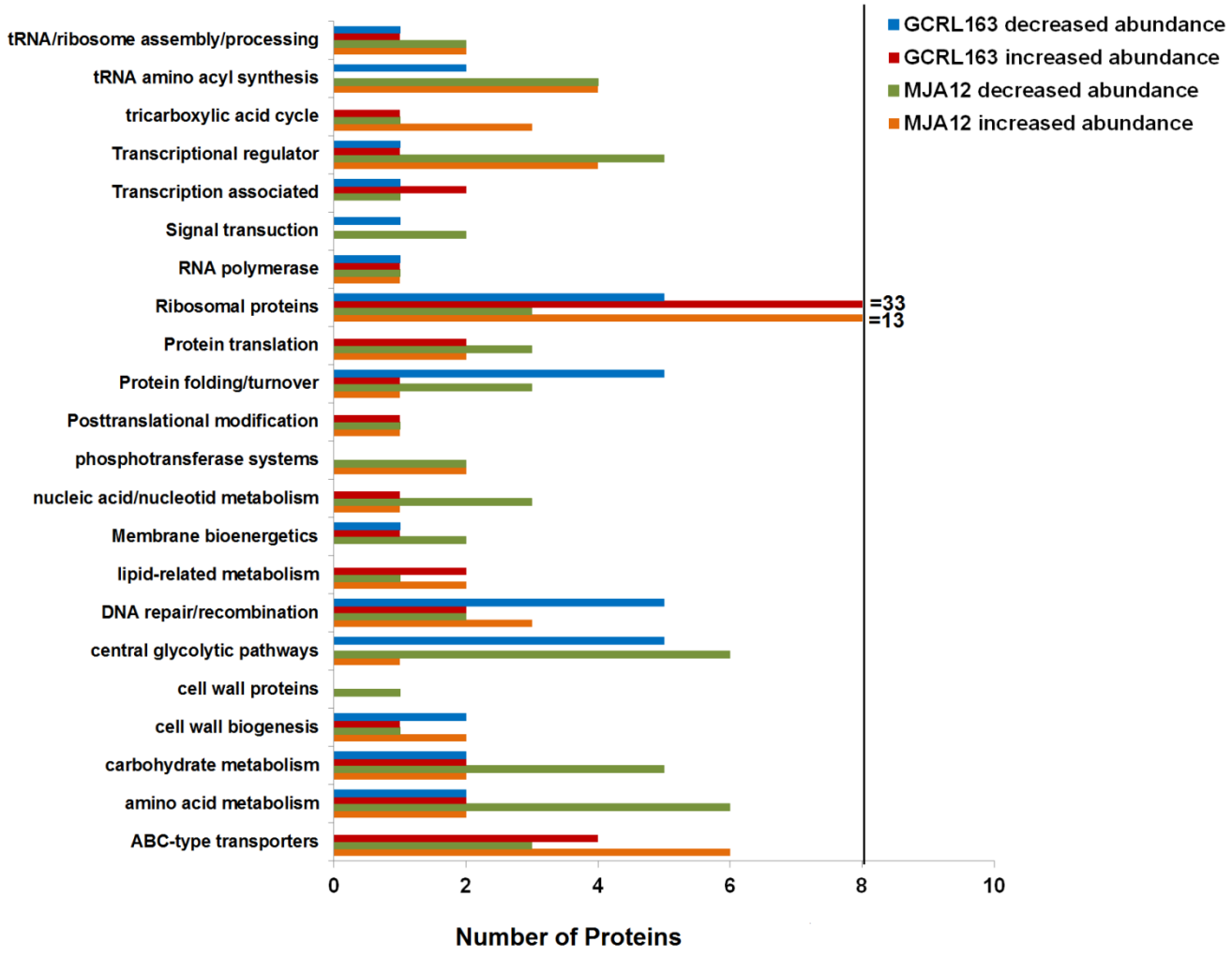


**Figure 3:** Enrichment ratio of whole cell relative to LiCl protein extracts for *Lb. casei* GCRL163 (Green) and MJA12 (Red).

**Table 2: Total spectral count of highly abundant proteins identified from LiCl extraction of *Lb. casei* GCRL163 and MJA12 strains at pH 6.5 and 4.5**

| | | | Average Spectral count | | | |
|---|---|---|---|---|---|---|
| **Protein name** | **Gene** | **NCBI ref no. (LC W56)** | **pH 6.5 GC** | **pH 4.5 GC** | **pH 6.5 MJ** | **pH 4.5 MJ** |
| phosphocarrier protein HPr | PtsH | YP_006752009 | 377.50 | 179.63 | 370.60 | 142.75 |
| DNA-binding protein HU | Hup/Hbs | YP_006751640 | 178.88 | 65.34 | 93.50 | 19.56 |
| elongation factor Tu | Tuf | YP_006751597 | 96.83 | 10.53 | 137.65 | 19.97 |
| Cold shock protein 1 | Csp | YP_006750765 | 92.59 | 14.79 | 26.31 | 2.20 |
| 30S ribosomal protein S16 | RpsP | YP_006751847 | 67.51 | 23.70 | 41.72 | 53.80 |
| DUF1447 superfamily protein | | YP_006751569 | 58.91 | 11.47 | 24.34 | 7.48 |
| 30S ribosomal protein S1 | RpsA | YP_006751638 | 58.16 | 7.11 | 69.79 | 35.71 |
| 50S ribosomal protein L7/L12 | RplL | YP_006752474 | 55.19 | 27.28 | 7.23 | 34.78 |
| 30S ribosomal protein S19 | RpsS | YP_006752684 | 54.82 | 37.31 | 29.84 | 18.86 |
| glyceraldehyde-3-phosphate dehydrogenase | Gap | YP_006751167 | 54.71 | 5.18 | 44.57 | 7.33 |
| 30S ribosomal protein S7 | RpsG | YP_006752694 | 51.56 | 44.10 | 8.70 | 42.92 |
| 50S ribosomal protein L16 | RplP | YP_006752681 | 50.86 | 7.49 | 2.18 | 10.61 |
| 30S ribosomal protein S10 | RpsJ | YP_006752689 | 45.43 | 5.53 | 18.12 | 4.12 |
| translation initiation factor IF-1 | InfA | YP_006752666 | 44.59 | 5.99 | 0.88 | 0.50 |
| 30S ribosomal protein S8 | RpsH | YP_006752674 | 44.14 | 4.21 | 33.78 | 3.51 |

| | | | | | | |
|---|---|---|---|---|---|---|
| hypothetical protein BN194_25890 | | YP_006752655 | 43.88 | 6.85 | 5.33 | 7.54 |
| 30S ribosomal protein S2 | RpsB | YP_006751834 | 38.47 | 4.50 | 31.84 | 12.23 |
| ABC-type transporter, substrate-binding protein | | YP_006750837 | 95.70 | 36.22 | 7.79 | 4.09 |
| 50S ribosomal protein L22 | RplV | YP_006752683 | 31.90 | 3.21 | 54.97 | 1.15 |
| DUF1831 superfamily protein | | YP_006751557 | 30.48 | 6.50 | 9.03 | 4.50 |
| hypothetical protein BN194_23630 / Cell wall-associated hydrolase | | YP_006752429 | 86.53 | 30.48 | 3.53 | 31.23 |
| 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase | GpmA2 | YP_006752340 | 28.84 | 271.25 | 56.29 | 202.63 |
| 50S ribosomal protein L27 | RpmA | YP_006751893 | 27.11 | 6.44 | 9.79 | 1.50 |
| Cold shock-like protein CspLA | CspLA | YP_006751312 | 26.75 | 0.50 | 12.47 | 0.50 |
| enolase | Eno | YP_006751170 | 25.44 | 19.37 | 86.00 | 16.73 |
| hypothetical protein BN194_01830 | | YP_006750249 | 24.53 | 18.47 | 1.95 | 2.85 |
| 30S ribosomal protein S21 | RpsU | YP_006751771 | 24.49 | 1.53 | 10.45 | 3.86 |
| 50S ribosomal protein L15 | RplO | YP_006752669 | 24.18 | 0.50 | 37.04 | 0.50 |
| tagatose 1,6-diphosphate aldolase 2 | LacD2 | YP_006752772 | 24.16 | 9.89 | 1.26 | 0.50 |
| 50S ribosomal protein L13 | RplM | YP_006752651 | 24.16 | 22.24 | 10.25 | 32.14 |
| 50S ribosomal protein L17 | RpsQ | YP_006752661 | 23.87 | 6.21 | 3.03 | 3.14 |
| 50S ribosomal protein L23 | RplW | YP_006752686 | 23.57 | 2.53 | 3.01 | 0.85 |
| acyl carrier protein | AcpP2 | YP_006752325 | 20.87 | 13.93 | 9.21 | 7.63 |
| catabolite control protein A | CcpA | YP_006750941 | 20.11 | 7.50 | 8.50 | 2.82 |

| | | | | | | |
|---|---|---|---|---|---|---|
| oligopeptide ABC-type transporter, substrate-binding protein | OppA2 | YP_006752130 | 19.77 | 10.79 | 6.06 | 3.18 |
| hypothetical protein BN194_24860 | | YP_006752552 | 18.50 | 7.24 | 39.27 | 1.49 |
| 50S ribosomal protein L29 | RpmC | YP_006752680 | 18.21 | 4.53 | 7.62 | 0.50 |
| 30S ribosomal protein S13 | RpsM | YP_006752664 | 18.14 | 0.50 | 16.64 | 0.85 |
| 30S ribosomal protein S4 | RpsD | YP_006751510 | 18.11 | 3.54 | 21.17 | 0.50 |
| fructose-bisphosphate aldolase | Fba2 | YP_006750572 | 16.54 | 2.82 | 1.82 | 1.18 |
| glycine cleavage system H protein | GcvH | YP_006751436 | 16.18 | 18.48 | 0.50 | 5.50 |
| 50S ribosomal protein L10 | RplJ | YP_006752475 | 16.16 | 8.47 | 11.06 | 2.15 |
| 50S ribosomal protein L18 | RplR | YP_006752672 | 15.47 | 32.37 | 1.20 | 1.48 |
| RNA-binding protein, S4_2 superfamily | YaaA | YP_006750070 | 14.78 | 3.47 | 15.07 | 3.15 |
| glycerol-3-phosphate ABC transporter substrate-binding protein | UgpB | YP_006751192 | 14.54 | 1.50 | 17.54 | 0.83 |
| 30S ribosomal protein S17 | RpsQ | YP_006752679 | 12.86 | 1.83 | 1.52 | 0.82 |
| xanthine phosphoribosyltransferase | Xpt | YP_006751315 | 12.81 | 1.50 | 1.20 | 0.50 |
| translation initiation factor IF-3 | InfC | YP_006751948 | 12.50 | 4.47 | 1.20 | 1.18 |
| 50S ribosomal protein L11 | RplK | YP_006752482 | 12.20 | 5.57 | 0.50 | 0.50 |
| hypothetical protein BN194_01870 | | YP_006750253 | 11.87 | 2.50 | 2.20 | 1.14 |
| 30S ribosomal protein S14 type Z | RpsZ | YP_006752675 | 11.49 | 2.17 | 2.56 | 2.17 |
| 50S ribosomal protein L24 | RpsX | YP_006752677 | 10.51 | 0.50 | 5.54 | 0.50 |
| 50S ribosomal protein L5 | RplE | YP_006752676 | 10.19 | 7.18 | 3.56 | 0.50 |

| | | | | | | |
|---|---|---|---|---|---|---|
| elongation factor G | Fus | YP_006752693 | 10.18 | 4.79 | 2.95 | 1.49 |
| Pur operon repressor | PurR2 | YP_006752762 | 9.84 | 20.80 | 3.53 | 1.51 |
| L-lactate dehydrogenase | Ldh | YP_006752722 | 9.82 | 8.86 | 4.39 | 2.51 |

**GC: *Lb. casei* GCRL163**

**MJ: *Lb. casei* MJA12**

**Lithium chloride extraction relative to whole cell extraction in terms of protein compartment bias.** Given that the significance of moonlighting proteins are a phenomena difficult to interpret directly, the LiCl extracts were examined more closely for the relative enrichment and depletion of proteins in different predicted subcellular compartments for both strains studied. The data for these observations are summarized in Figure 5. The results suggest LiCl extracts contained a relatively consistent but modest enrichment of transmembrane proteins 180-190% higher than for lysates obtained by bead beading while membrane-associated proteins and putative secreted proteins are more variably enriched between the strains examined (from 106 to 200%). Cell wall-specific proteins, mostly those that are sorted and linked to peptidoglycan and usually also associated with the cytoplasmic membrane (via a conserved domain region or regions), were only weakly enriched (135%). Cytosolic proteins were by comparison only weakly depleted (20% reduction) overall.

Most proteins identified in the datasets in this study have relatively low abundance and thus the ability to accurately estimate ratios between the extraction protocols is challenging. Many proteins could be present in the LiCl extract merely due to cell lysis, noting that even minor cell lysis followed by concentrating extracts 100-fold to detect proteins would amplify the level of cytosolic protein contamination in the extract. Furthermore, growth a low pH would favour binding of released protein back onto cells (see Kainulainen and Korhonen, 2014). Nevertheless the differential extraction of proteins between strains GCRL163 and MJA12 strongly correlated (0.68, $p<0.001$, Figure 4), indicating LiCl extraction in general affects the strains similarly.

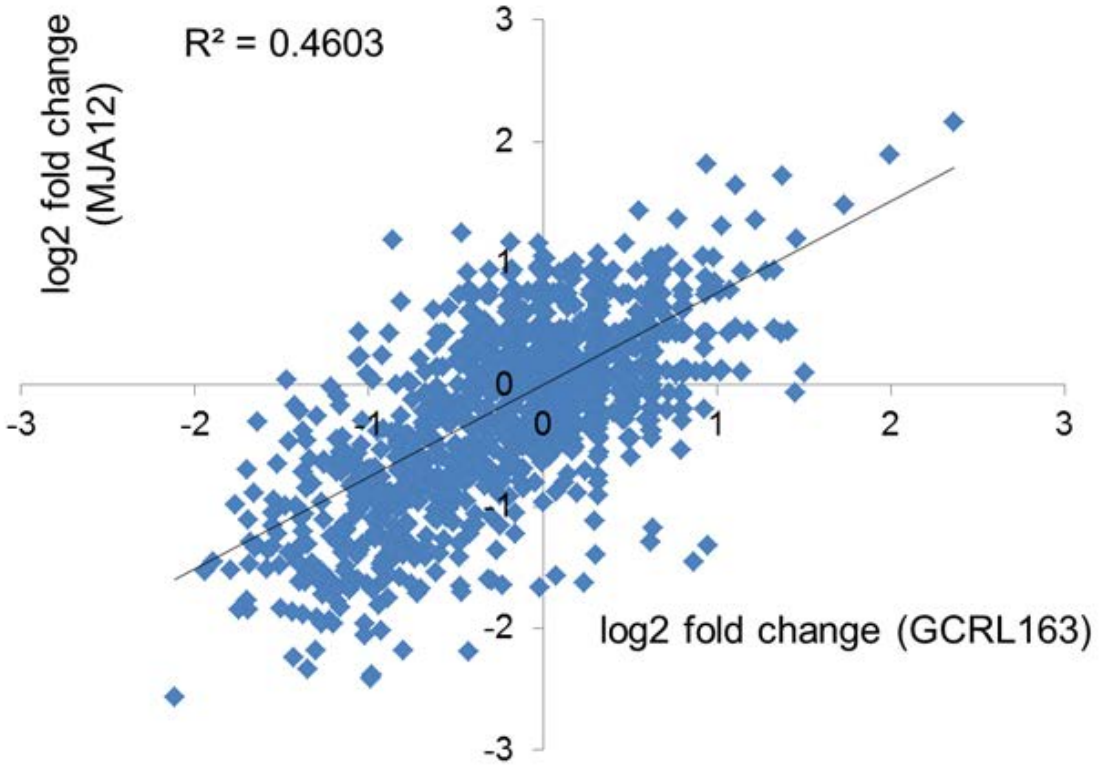


**Figure 4:** Correlation of enriched proteins of *Lactobacillus casei* GCRL163 vs MJA12 extracted using 5 M LiCl.

**Highly depleted and enriched proteins obtained following LiCl extraction.**

Depleted proteins tended to include those that are cytosolically compartmentalised and possess relatively high cellular abundances. These included proteins that are often observed *via* 2D-SDS gel electrophoresis and have important cellular roles, such as GroEL, DnaK, RpoB, RpoC, various ribosomal and sugar metabolism-related proteins (Appendix D). Only one depleted protein (RmlB2), likely associated with synthesis of capsular polysaccharide, had a predicted membrane helical domain. Enriched proteins included mainly those that have predicted membrane associations, which are putatively trafficked within and/or out of the cell or are also mainly cytosolic in nature. The list includes several proteins that have a role in cell wall biogenic processes including peptidoglycan crosslinking, turnover and modification (PgdA, PonA, LytF) and the regulation of these processes (LytR proteins). It would be expected these proteins are localised at the cell wall. Two enriched, rather poorly conserved proteins annotated as cell wall hydrolases lack detectable conserved domains so their functional assignation must be considered with caution. One protein with a LPTXG motif indicative of a peptidoglycan anchor domain was clearly significantly enriched. This particular cell wall protein is not conserved in all *Lb. casei* strains: whether this protein has any specific significance to adhesion is not known. Two membrane-associated proteins with von Willebrand factor type A domains, typically found in proteins associated in complexes, were enriched. The function and association of these proteins in *Lb. casei* is currently not known. In addition, a phosphotransferase type transporter protein was observed to be enriched though it represented the inner membrane positioned subunit (Appendix D). Several proteins with no known function were enriched while small cytosolic proteins were also enriched, in particular the conserved (though *Firmicutes* specific) S4 domain protein YaaA (*B. subtilis* designation), which putatively binds RNA but otherwise has an unclear

cellular role. The overall data suggests that LiCl extraction is not specific and enhances detection of various proteins such as various membrane proteins. However, the effects of LiCl are not clear cut and it is possible LiCl extraction also differentially affects some proteins over others based on their physical properties not location, such as certain low molecular weight proteins.

**Acid adapted cell derived protein profiles.**

Proteins that show largely increased and decreased abundance trends in common between strains GCRL163 and MJA12 that have become acid adapted are shown in Table 1 while numbers of these proteins associated with functional groups are shown in Figure 5. A high proportion of proteins that become more abundant were ribosomal proteins, 33 for MJA12 and 13 for GCL163. The only other functional group that produced a substantial number of proteins of greater abundance includes ABC-type transporter subunit proteins. This set comprised ABC-type transporter subunits associated with uptake of compatible solutes (glycine-betaine, choline), oligopeptides, and unknown solutes. Protein folding and turnover and central glycolytic pathways and carbohydrate-related metabolism included the greatest proportion of proteins with reduced abundance. A total of 148 proteins showed differential responses between the strains. This could be attributable partly to the signal to noise in the data. Approximately one-third of these proteins show large differences between strains (at least 5-fold in terms of relative abundance). Most of these proteins were associated with protein synthesis and carbohydrate uptake, metabolism and fermentation (Table 1). A variety of cell membrane associated proteins noted to be enriched in the LiCl extracts in comparison to whole cell extracts are amongst this subset. One peptidoglycan anchored protein was abundant in MJA12 cells grown at pH 6.5 but

disappeared in cells adapted to pH 4.5, however the equivalent homolog in GCRL163 showed no significant changes in abundance.

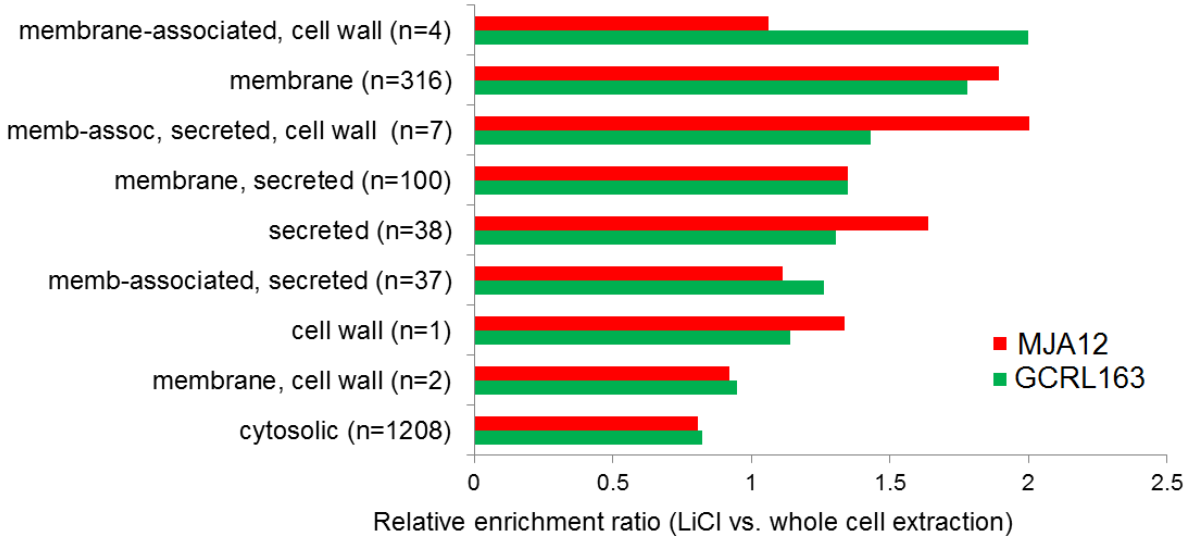


**Figure 5:** Number of spectra counts of protein identified in LiCl extracts in the current study associated with different functions.

**Discussion**

Surface-associated and surface-anchored proteins play a critical role in adhesion and subsequent colonization of probiotic bacteria in the GIT. LiCl extracts were analysed using nano-LC-LTQ-Orbitrap tandem mass spectrometry and spectral counting, which provides broad protein coverage and enables relative quantification. The proteomic data shows that the extracts contained proteins that were either enriched or depleted relative to their presence in cell-free lysate extracts. The highly enriched proteins in these extracts included transmembrane proteins, membrane-associated proteins, extracellular secreted proteins and cell wall specific proteins. Cell wall properties are critical for adhesion and likely involve the interaction of a number of factors, including surface proteins that can have specific and non-specific binding capacities as well as the inherent hydrophobicity or electrostatic properties. Suspected proteins involved in adhesion have been delineated on the basis of conserved domains or determined experimentally. Azcarate-Peril *et al*. (2008) showed that *Lb. gasseri* ATCC 33323 contains 14 mucus-binding proteins, six of which were likely secreted and four of which were predicted to be covalently linked to the membrane *via* sortase A. It was also shown that these proteins are involved in adhesion of *Lb. reuteri* 1063 to mucin (Roos *et al*., 2002) and *Lb. acidophilus* NCFM adhesion to Caco-2 cells (Buck *et al*., 2005).

GAPDH and enolase have been identified on the outer surface of *Lb. crispatus* and other *Lactobacillus* strains (Antikainen *et al*., 2007; Kinoshita *et al*., 2008; Beck *et al*., 2009) and detected in mildly alkaline buffer containing intact cells. Glenting *et al*. (2013) showed that GAPDH and enolase isolated from the outer membrane of *Lb. plantarum* 299v had demonstrable binding to plasminogen, fibronectin, and in the case of GAPDH to mucin. Furthermore, they also

showed that the GAPDH and enolase binding to Caco-2 cells was pH dependent. In this respect their findings agree with our data analysis where both *Lb. casei* strains shows better attachment to HT-29 epithelium cell when *Lb. casei* adapted at pH 4.5, possibly due to low pH favouring binding to bacterial cells. GAPDH and enolase were also detected in LiCl extracts here (Table 1), with the total spectral count for GAPDH increasing in strain MJA12 following growth at pH4.5. The glycolytic enzymes enolase and GAPDH are also found on the surface of several Gram-positive bacteria, where they can be involved in pathogen host interactions (Bergmann *et al*., 2004; Boël *et al*., 2005). Hurmalainen *et al*. (2007) showed that commensal *Lb. crispatus* and several other species of the acidophilus group of *Lactobacillus* have enolase and GAPDH as major constituents of their extracellular proteome at neutral pH extracellular proteome at neutral pH. However it is important to say that it is possible *many* other proteins could also interact with host glycoproteins, such as mucin, and with glycoprotein-rich surfaces of epithelial cells. Until many other proteins, or combinations of these, are specifically tested, as well as gene deletion mutants lacking certain cell wall associated proteins, can any progress be made in delineating which proteins are specifically associated with adhesion and host cell-interactions.

A previous study by Lortal *et al*. (1991), showed that using LiCl to extract the S-layer proteins from *Lb. helveticus* was apparently an efficient method, where bacterial cell viability did not decrease significantly and extracted proteins had a high content of hydrophobic amino acids. In addition, Sanchez *et al*. (2009) used six different methods to extract the surface-associated proteins from *Lb. rhamnosus* GG, where treatment with 5 M LiCl appearing to be the most suitable for surface-associated protein extraction. In the strains of *Lb. casei* tested in this study, LiCl extraction demonstrated that differences in culture conditions led to qualitative and

quantitative differences in extractable proteins, including known moonlighting proteins involved in glycolysis (enolase, GAPDH, tagatose 1,6-biphosphae aldolase 2, L-lactate dehydrogenase) and a hypothetical protein with possible functionality as a hydrolase/carboxy peptidase/penicillin binding protein, which were either abundant in the extracts or altered in relative proportions following growth at low pH. It is probably not surprising to observe that there appears to be considerable strain to strain variation in the surface proteins seen in *Lb. casei* strains from different ecological sources. Based on the adhesion data here, LiCl extracted proteins have a physical effect on the *Lb. casei* adhesive capacity, indicating that the proteins in the extract were important for bacterial cell binding. Using a suite of cell fractionation approaches may prove fruitful in identifying the relative importance of various surface proteins in adhesion.

In conclusion, this study presents the proteomic analysis using LiCl extraction method for *Lb. casei* GCRL163 and MJA12 strains grown at neutral and low pH. Growth of *Lb. casei* at low pH enhanced the adherence properties of GCRL163 and MJA12 strains. Adding the extracted cell surface proteins to HT-29 cell decreased the number of adhered bacterial cells, which suggests that the adhesion of GCRL163 and MJA12 might relate to some of these proteins. Treatment of *Lb. casei* with 5 M LiCl enriched or depleted different proteins and demonstrated that the ability of LiCl to fractionate cell surface proteins was very poor. Further research may be helpful to compare these LiCl extraction results with other methods like trypsin shaving (Tjalsma *et al*., 2008) which is another major technique used to demonstrate the location of cell wall proteins. Further research focusing of the adhesion of *Lb. casei* in the presence of specific proteins in cellular fractions to HT-29 will be helpful in understanding the adhesion mechanisms of *Lb. casei*.

# CHAPTER 6

# GENERAL CONCLUSION

This study was initiated to investigate the physiological responses of *Lb. casei* strains under various stress conditions relevant to survival in fermented foods such as cheese, fermented milk and yoghurt. Research focused on responses to carbohydrate starvation and acid growth adaptation with investigations primarily performed using a relative quantitative proteomic approach (nano-LC-LTQ-Orbitrap tandem mass spectrometry). This research provided complementary information to that already available in the literature and allowed growth responses observed to be deconstructed to the level of metabolic pathways and proteins. This strategy has been used in several other studies in relation to food-associated bacteria and has proven to be a useful tool and gives a provides a different perspective on complex biological systems (Rauch *et al*., 2006; Searle, 2010; Bowman *et al*., 2012).

In Chapter 2, published in the Journal of Proteome Research (Al-Naseri *et al*., 2013) the study focused on carbohydrate stress. This involved determining the proteomic differences between *Lb. casei* GCRL163 grown in the presence of three different lactose concentrations (0% starvation, 0.2% limited growth, 1% control). In addition with presence and absence of the surfactant Tween 80 was also considered since it may act as an alternative carbon source (see below). The proteomic data shows that the absence of lactose "starvation" *Lb. casei* GCRL163 induces several pathways targeted at cellular energy production, essentially a scavenging-like response. In particular the proteomic data demonstrated increases in the abundance of proteins related to the citrate fermentation pathway, which consists of seven proteins making up the

citrate lyase (CitXFED) and oxaloacetate decarboxylase (OadAB) complexes. Increased abundance of enzymes making up pathways for the catabolism of polyols, including inositol and glycerol, as well as other sugars, possibly present in the medium at low levels (for example in yeast extract) was also evident. It was observed that the presence of Tween 80 affected the abundance of these proteins markedly with citrate fermentation enzymes strongly promoted while carbohydrate scavenging-related proteins only promoted significantly in the absence of Tween 80. End-product analysis (using GC-MS) of cultures grown in the presence of Tween 80 demonstrated an increase in octanoic acid and caproic acid levels compared with controls (0% lactose no Tween 80), suggesting that GCRL163 has the ability to weakly degrade Tween 80 with resultant metabolites potentially influencing cell physiology. This manifested as apparent suppression of fatty acid biosynthethic pathway though the basis of this effect is unclear, noting that the medium contained citrate and this probably repressed use of Tween 80 and other carbon sources present in the medium.

Viability of *Lb. casei* dietary adjuncts is important in order to provide health benefits. This study suggests that the absence of the main carbon source in the media resulted in high viability of bacterial cells after 30 days. This was partially explained as controls with lactose contained high levels of lactate which would have impacted on cell viability over time. The industry always aims to have high numbers of viable bacteria in food products that do not affect product flavor. Prior growth conditions of probiotics being added to food products or prepared as pharmaceuticals will be an important factor in influencing subsequent survival and probiotic functionality.

As a result of the chapter 2 experiments and the intriguing results surrounding Tween 80, a set of experiments was carried out to further characterize the growth of *Lb. casei* GCRL163 with Tween 80, in the presence and absence of citrate. The results are described in Chapter 3. The growth kinetics and proteomic data further suggests that strain GCRL163 can grow directly on Tween 80 possibly, attacking the sorbitan section of the molecule. This was surmised since GCRL163, like most *Lactobacillus* strains, lacks the ability to catabolise fatty acids, since the required enzymes for anaerobic fatty acid metabolism proteins (FadI, FadJ, FadK) are absent. Cultures grown with 0.1% Tween 80 increased abundance of proteins involved in pathways related to glycerol metabolism and fatty acid biosynthesis. The metabolism of Tween 80 appears to have a substantial effect on cell physiology resulting in a "hardening", providing protection against acid shock and prolonged acid pH (pH 2) exposure. Based on the proteomic analysis, this phenotype arises from promotion of fatty acid biosynthesis; protein folding turnover and disaggregation; polar amino acid accumulation, and possibly stimulation of compatible solute uptake. Tween 80's ability to enhance the survival of *Lb. casei* GCRL163, including greater tolerance to lethal acidic conditions, was also linked to possible changes in the surface properties of *Lb. casei* GCRL163 potentially linked to markedly more abundant cell surface proteins. The data shown here contributes to explaining the effect of Tween 80 on the physiology of GCRL163.

Given that *Lb. casei* with its active carbohydrate fermentative capacity is comparatively aciduric, a further study was implemented to examine the growth of *Lb. casei* GCRL163 and MJA12 strain at low pH as described in Chapter 4. This was to discern what means the species uses to defend against acidic pH. Furthermore, two strains were tested to determine the

consistency of such responses at the strain level. Experimentally, the two strains investigated were adapted to grow at pH 4.5 (low pH) and pH 6.5 (the control) within a fermentor system where anaerobic conditions and stable pH could be maintained. The data suggested that the two *Lb. casei* strains show responses that are growth phase, pH, and strain dependent, with the growth phase being most influential overall. Intracellular accumulation of amino acids seems quite active and thus an exogenous supply of protein likely could aid long term survival in acidic foods, this strategy is also used by other *Lactobacillus* strains to withstand various environmental stresses (Wu *et al*., 2012; Wu *et al*., 2013). Enzymes related to membrane bioenergetics, end-product formation and energy yielding catabolic pathways show different abundances under the growth conditions examined reflecting adaptation to different energetic demands exerted by pH effects. The proteomic data suggests that fermentation metabolism was different between the strains tested, L-lactate and D-lactate dehydrogenases were more abundant at stationary phase for strain MJA12, however, these enzymes were not significantly altered in abundance in GCRL163. Other groups of enzymes involved in citrate and malolactic fermentation were more abundant for GCRL163 and MJA12 strains, respectively. Protein abundance data also revealed that acid adaptation varied between the strains in a growth phase-dependent manner, with strain MJA12 exhibiting most known acid adaptation-type responses when in the stationary growth phase, unlike strain GCRL163 which seems more directly responsive to acidic pH conditions with responses more observed in the exponential growth phase. The results presented expand the knowledge on how lactic acid bacteria respond to acid stress and strongly suggests further studies should include multiple strains to account for strain-dependent behaviour.

Further research was employed to investigate the impact of low pH on *Lb. casei* surface associated proteins and the adhesion properties of GCRL163 and MJA12 strains, described in Chapter 5. Growth of *Lb. casei* at low pH was found to enhance the adherence of GCRL163 and MJA12 strains to cultured HT29 cells. Interaction of some of these proteins with the HT-29 cell line might be part of probiotic traits exhibited by *Lb. casei*. The LiCl extraction method was able to demonstrate changes in abundance of proteins comparative to their abundance in cell lysates and changes in abundance following growth at different pHs. Further work with alternative methods for selectively fractionating cell surface proteins are required to advance this particular area of research.

**Future Research**

Gel-free proteome-based analysis provided a comprehensive insight into the functional changes and physiology responses of *Lb. casei* during carbon starvation and growth at low pH. Further study could provide further clarity and depth of understanding into these responses since they are key to *Lb. casei* being useful as a general food adjunct. The following topics were identified as potentially useful areas for specific study:

1. More research into identify specific substrate uptake by *Lb. casei* under carbohydrate starvation and more specific is identify unknown protein that has high abundant when lactose was absent in mMRS, that will help to understand how *Lb. casei* regulates carbon source utilisation and multiauxic responses. Changes in relative levels of expression of proteins related to carbohydrate or other substrate raises the question about how cells with metabolism and what other substrate that it can use in mMRS to survive and maintain viability.

2. A focused effort to determine how adhesion properties of *Lb. casei* in the presence or absence of Tween 80 are affected and the molecular basis of adhesion. The proteomic data show changes on the cell surface properties of *Lb. casei* and show increase in abundant of these proteins when Tween 80 presence in mMRS. A further study to look at the differences in adhesion of *Lb. casei* with presence of Tween 80 will improve the understanding of this mechanism.

3. Studying the application of Tween 80 or other food grade surfactants that may be a useful means to adjust probiotic physiological properties, enhancing survival in food or non-food delivery systems and subsequently passage into the host intestinal environment. Understanding *Lb. casei* respond under single type of stress was helpful to understand the mechanism of surviving but understanding regulatory mechanisms in *Lb. casei* under different stress in one experiment e.g. carbohydrate starvation, as well as, lacking of Tween 80, and acid stress can give better understanding of the mechanism of adaptation under multi type of stress and that should allow us to understand the adaptation responses and help to present an appropriate *Lb. casei* for industry use.

4. Studying the effect of low pH at the metabolomics level with absolute of metabolite concentrations deserves attention, especially in relation to strain variation. Such knowledge will improve the predictability of the metabolism of probiotics applied in food systems.

5. Surface proteins are likely better extracted with methods other than LiCl extraction, for example, applying trypsin shaving to identify surface proteins (Cathy *et al*., 2013) and to explore the quantitative changes in the surface.

6. Proteomics will provide improved knowledge of how surface proteins contribute to adhesion and also the degree that other proteins associated with the cell surface, such as moonlighting

proteins including GAPDH and enolase, as well as lectin-binding proteins, might contribute to probiotic cell biology in *Lb. casei*. The LiCl extracts fron cells grown under low pH contain many proteins that may be related to increasing the number of bacterial cells adhering to HT-29. Further work, for example, could include adding specific proteins to HT-29 monlayers prior to determining the level of *Lb. casei* adhesion. More specifically, using proteins which have high spectral counts from LiCl extracts following growth at low pH. This type of approach may identify new proteins that may involve in *Lb. casei* adhesion in addition to the known proteins identified to date.

The data shown in the thesis contributes to explaining the adaptations of *Lb. casei* to some food-relevant stress scenarios, however clearly much further work is needed to develop a more complete and refined knowledge on the behavior of lactic acid bacteria in food systems, especially those intended as dietary adjun*cts.* Advanced approaches, including metabolomics, quantitative proteomics and cell biology, when integrated would provide powerful means to develop this area further.

## REFERENCES


Adamberg, S., Sumeri, I., Uusna, R., Ambalam, P., Kondepudi, K. K., Adamberg, K., Wadström, T. and Ljungh, Å. (2014). Survival and synergistic growth of mixed cultures of bifidobacteria and lactobacilli combined with prebiotic oligosaccharides in a gastrointestinal tract simulator. Microbial Ecology in Health and Disease, 25, 1-9.

Adlerberth, I., Ahrne, S., Johansson, M. L., Molin, G., Hanson, L. A. and Wold, A. E. (1996). A mannose-specific adherence mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. Applied and Environmental Microbiology. 62, 2244-2251.

Akçelik, N., Şimşek, Ö. and Akçelik, M. (2014). Recent Advances in Genetics of Lactic Acid Bacteria. Dairy Microbiology and Biochemistry: Recent Developments, 68-85.

Alam, M., Midtvedt, T. and Uribe, A. (1994). Differential Cell-Kinetics in The Ileum and Colon of Germ-Free Rats. Scandinavian Journal of Gastroenterology, 29, 445-451.

Anderson, M.J. and Willis T.J. (2003). Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. Ecology, 84, 511-525.

Antikainen, J., Anton, L., Sillanpaa, J. and Korhonen, T. K. (2002). Domains in the S-layer protein CbsA of *Lactobacillus crispatus* involved in adherence to collagens, laminin and lipoteichoic acids and in self-assembly. Molecular Microbiology. 46, 381-394.

Antikainen, J., Kuparinen, V., Lahteenmaki, K., and Korhonen, T.K. (2007) pH-dependent association of enolase and glyceraldehyde-3-phosphate dehydrogenase of *Lactobacillus crispatus* with the cell wall and lipoteichoic acids. Journal of Bacteriology, 189, 4539–4543.

Antonio, M. A., Rabe, L. K., & Hillier, S. L. (2005). Colonization of the rectum by *Lactobacillus* species and decreased risk of bacterial vaginosis. Journal of Infectious Diseases, 192, 394-398.

Anyogu, A., Awamaria, B., Sutherland, J. and Ouoba, L. (2014). Molecular characterisation and antimicrobial activity of bacteria associated with submerged lactic acid cassava fermentation. Food Control. 39, 119-127.

Ardo, Y. (2006). Flavour formation by amino acid catabolism. Biotechnology Advances. 24, 238-242.

Avall-Jaaskelainen, S., and Palva, A. (2005). *Lactobacillus* surface layers and their applications. FEMS Microbiology Reviews, 29, 511-529.

Åvall-Jääskeläinen, Silja, and Airi Palva, A. (2005). *Lactobacillus* surface layers and their applications. FEMS Microbiology Reviews, 29, 511-529.

Awad, S., Ahmed, E., El, Soda, M. (2007). Evaluation of isolated starter lactic acid bacteria in Ras cheese ripening and flavour development. Food Chemistry. 104, 1192–1199.

Azcarate-Peril, M. A., Altermann, E., Goh, Y. J., Tallon, R., Sanozky-Dawes, R. B., Pfeiler, E. A., O'flaherty, S., Buck, B. L., Dobson, A., Duong, T., Miller, M. J., Barrangou, R. and Klaenhammer, T. R. (2008). Analysis of the genome sequence of *Lactobacillus gasseri* ATCC 33323 reveals the molecular basis of an autochthonous intestinal organism. Applied and Environmental Microbiology, 74, 4610-4625.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. and Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. Science, 307, 1915-1920.

Bang, M., Oh, S., Lim, G. S., Kim, Y., and Oh, S. (2014). The involvement of ATPase activity in the acid tolerance of *Lactobacillus rhamnosus* strain GG. International Journal of Dairy Technology. 67, 229-236.

Beaufils, S., Sauvageot, N., Mazé, A., Laplace, J.-M., Auffray, Y., Deutscher, J. and Hartke, A. (2006). The cold shock response of *Lactobacillus casei*: relation between HPr phosphorylation and resistance to freeze/thaw cycles. Journal of Molecular Microbiology and Biotechnology. 13, 65-75.

Beck, H. C., Madsen, S. M., Glenting, J., Petersen, J., Israelsen, H., Nørrelykke, M. R., and Hansen, A. M. (2009). Proteomic analysis of cell surface-associated proteins from probiotic *Lactobacillus plantarum*. FEMS Microbiology Letters. 297, 61-66.

Bender, G. R., and Marquis, R. E. (1987). Membrane Atpases and acid tolerance of *Actinomyces viscosus* And *Lactobacillus casei*. Applied and Environmental Microbiology, 53, 2124-2128.

Beresford, T. P., Fitzsimons, N. A., Brennan, N. L., and Cogan, T. M. (2001). Recent advances in cheese microbiology. International Dairy Journal, 11, 259-274.

Bergmann, S., M. Rohde, and S. Hammerschmidt. (2004). Glyceraldehyde-3- phosphate dehydrogenase of *Streptococcus pneumoniae* is a surface-displayed plasminogen-binding protein. Infect. The Journal of Immunology. 72, 2416- 2419.

Bernet, M. F., Brassart, D., Neeser, J. R., and Servin, A. L. (1994). *Lactobacillus acidophilus* La-1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut, 35, 483-489.

Beveridge, T. J., and Graham, L. L. (1991). Surface layers of bacteria. Microbiological Reviews, 55, 684-705.

Bisanz, J. E., and Reid, G. (2011). Unraveling how probiotic yogurt works. Science Translational Medicine, 3, 106ps41-106ps41.

Boekhorst, J., Helmer, Q., Kleerebezem, M., and Siezen, R. J. (2006). Comparative analysis of proteins with a mucus-binding domain found exclusively in lactic acid bacteria. Microbiology-SGM, 152, 273-280.

Boël, G., H. Jin, and V. Pancholi. (2005). Inhibition of cell surface export of group A *Streptococcal anchorless* surface dehydrogenase affects bacterial adherence and antiphagocytic properties. Infect. The Journal of Immunology. 73, 6237- 6248.

Bongaerts, G. P. A., and Severijnen, R. (2001). The beneficial, antimicrobial effect of probiotics. Medical Hypotheses, 56, 174-177.

Boon, E., Meehan, C. J., Whidden, C., Wong, D. H. J., Langille, M. G., and Beiko, R. G. (2014). Interactions in the microbiome: communities of organisms and communities of genes. FEMS Microbiology Reviews, 38, 90-118.

Boorsma, A., Foat, B.C., Vis, D., Klis, F., Bussemaker, H.J. (2005). Tprofiler: scoring the activity of predefined groups of genes using gene expression data. Nucleic Acids Res. 33, 592−595.

Boot, H.J., Kolen, C.P., Andreadaki, F.J., Leer, R.J., Pouwels, P.H. (1996b). The *Lactobacillus acidophilus* S-layer protein gene expression site comprises two consensus promoter sequences, one of which directs transcription of stable mRNA. Journal of Bacteriology. 178, 5388–5394.

Booth, I. R. (1985). Regulation of cytoplasmic pH in bacteria. Microbiological Reviews, 49, 359-378.

Bowman, J. P., Hages, E., Nilsson, R. E., Kocharunchitt, C., and Ross, T. (2012). Investigation of the *Listeria monocytogenes* Scott A acid Tolerancetresponse and associated physiological and phenotypic features via whole proteome analysis. Journal of Proteome Research, 11, 2409-2426.

Bowman, J., Brul, S., Frataminco, P. M., and McMeekin, T. A. (2010). Protein-based analysis and other new and emerging non-nucleic acid based methods for tracing and investigating foodborne pathogens tracing pathogens in the food chain (pp. 292-341). Cambridge UK: Woodhead Publishing.

Broadbent, J. R., Larsen, R. L., Deibel, V., and Steele, J. L. (2010). Physiological and transcriptional response of *Lactobacillus casei* ATCC 334 to acid stress. Journal of Bacteriology, 192, 2445-2458.

Broadbent, J. R., Neeno-Eckwall, E. C., Stahl, B., Tandee, K., Cai, H., Morovic, W., Horvath, P., Heidenreich, J., Perna, N. T., Barrangou, R. and Steele, J. L. (2012). Analysis of the *Lactobacillus casei* supragenome and its influence in species evolution and lifestyle adaptation. BMC Genomics, 13, 1-18.

Buck, B. L., Altermann, E., Svingerud, T., and Klaenhammer, T. R. (2005). Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. Applied and Environmental Microbiology, 71, 8344-8351.

Buck, J. D., and Cleverdon, R. C. (1961). The Effect of Tween 80 on the enumeration of marine bacteria by the spread and pour plate methods. Limnology and Oceanography, 6, 42-44.

Cai, H., Thompson, R., Budinich, M. F., Broadbent, J. R., and Steele, J. L. (2009). Genome sequence and comparative genome analysis of *Lactobacillus casei*: insights into their niche-associated evolution. Genome Biology and Evolution, 1, 239-257.

Canchaya, C., Claesson, M. J., Fitzgerald, G. F., Van Sinderen, D., and O'Toole, P. W. (2006). Diversity of the genus *Lactobacillus* revealed by comparative genomics of five species. Microbiology, 152, 3185-3196.

Candela, M., Perna, F., Carnevali, P., Vitali, B., Ciati, R., Gionchetti, P., Rizzello, F., Campieri, M. and Brigidi, P. (2008). Interaction of probiotic *Lactobacillus* and *Bifidobacterium* strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production. International Journal of Food Microbiology, 125, 286-292.

Carasi, P., Ambrosis, N. M., De Antoni, G. L., Bressollier, P., Urdaci, M. C., and Serradell, M.D.L.A. (2014). Adhesion properties of potentially probiotic *Lactobacillus kefiri* to gastrointestinal mucus. Journal of Dairy Research, 81, 16-23.

Carlsson, J. (1997). Bacterial metabolism in dental biofilms. Advances in dental research, 11, 75-80.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., and Gibbs, P. (2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria. International Dairy Journal, 14, 835-847.

Zhang, C. X., Creskey, M. C., Cyr, T. D., Brooks, B., Huang, H., Pagotto, F., and Lin, M. (2013). Proteomic identification of *Listeria monocytogenes* surface-associated proteins. Proteomics, 13(20), 3040-3045.

Champagne, C. P., Gardner, N. J., and Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. Critical Reviews in Food Science and Nutrition, 45, 61-84.

Champomier-Verges, M. C., Maguin, E., Mistou, M. Y., Anglade, P., and Chich, J. F. (2002). Lactic acid bacteria and proteomics: current knowledge and perspectives. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 771, 329-342.

Chandry, P. S., Moore, S. C., Davidson, B. E., and Hillier, A. J. (1998). Investigation of the microbial ecology of maturing cheese by PCR and PFGE. Australian Journal of Dairy Technology, 53, 117.

Chatterji, D., and Ojha, A. K. (2001). Revisiting the stringent response, ppGpp and starvation signaling. Current Opinion in Microbiology, 4, 160-165.

Chauviere, G., Coconnier, M. H., Kerneis, S., Fourniat, J., and Servin, A. L. (1992). Adhesion of human *Lactobacillus acidophilus* strain-Lb to human enterocyte like Caco-2 Cells. Journal of General Microbiology, 138, 1689-1696.

Chen, C., Ai, L., Zhou, F., Wang, L., Zhang, H., Chen, W., Guo, B. (2011). Complete genome sequence of the probiotic bacterium *Lactobacillus casei* LC2W. Journal of Bacteriology. 193, 3419-3420.

Chen, X., Xu, J., Shuai, J., Chen, J., Zhang, Z., & Fang, W. (2007). The S-layer proteins of *Lactobacillus crispatus* strain ZJ001 is responsible for competitive exclusion against *Escherichia coli* O157: H7 and *Salmonella typhimurium*. International Journal of Food Microbiology, 115, 307-312.

Chu, H., and Mazmanian, S. K. (2013). Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nature Immunology, 14, 668-675.

Claesson, M. J., van Sinderen, D., and O'Toole, P. W. (2007). The genus *Lactobacillus* a genomic basis for understanding its diversity. FEMS Microbiology Letters, 269, 22-28.

Coconnier, Marie Helene, T. R. Klaenhammer, S. Kerneis, M. F. Bernet, and A. L. Servin. (1992). Protein-mediated adhesion of *Lactobacillus acidophilus* BG2FO4 on human enterocyte and mucus-secreting cell lines in culture. Applied and Environmental Microbiology 58, 2034-2039.

Coeuret, V., Dubernet, S., Bernardeau, M., Gueguen, M., and Vernoux, J. P. (2003). Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Le Lait, 83, 269-306.

Cohen, D. P. A., Renes, J., Bouwman, F. G., Zoetendal, E. G., Mariman, E., de Vos, W. M., and Vaughan, E. E. (2006). Proteomic analysis of log to stationary growth phase *Lactobacillus plantarum* cells and a 2-DE database. Proteomics, 6, 6485-6493.

Collado, M. C., Grzeskowiak, L., and Salminen, S. (2007). Probiotic strains and their combination inhibit in vitro adhesion of pathogens to pig intestinal mucosa. Current Microbiology, 55, 260-265.

Collins, M., Rodrigues, U., Ash, C., Aguirre, M., Farrow, J., Martinez-Murcia, A. and Wallbanks, S. (1991). Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. FEMS Microbiology Letters, 77, 5-12.

Coman, M. M., Verdenelli, M. C., Cecchini, C., Silvi, S., Orpianesi, C., Boyko, N., and Cresci, A. (2014). In vitro evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501®, *Lactobacillus paracasei* IMC 502® and SYNBIO® against pathogens. Journal of Applied Microbiology, 2, 518-527.

Corcoran, B. M., Stanton, C., Fitzgerald, G. F., and Ross, R. P. (2007). Growth of probiotic lactobacilli in the presence of oleic acid enhances subsequent survival in gastric juice. Microbiology-SGM, 153, 291-299.

Corcoran, B. M., Stanton, C., Fitzgerald, G., and Ross, R. P. (2008). Life under stress: the probiotic stress response and how it may be manipulated. Current Pharmaceutical Design, 14, 1382-1399.

Corcoran, B., Stanton, C., Fitzgerald, G., and Ross, R. (2005). Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. Applied and Environmental Microbiology, 71, 3060-3067.

Corr, S. C., Li, Y., Riedel, C. U., O'Toole, P. W., Hill, C., and Gahan, C. G. (2007). Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proceedings of the National Academy of Sciences, 104, 7617-7621.

Cotter, P. D., and Hill, C. (2003). Surviving the acid test: Responses of Gram-positive bacteria to low pH. Microbiology and Molecular Biology Reviews, 67, 429-453.

Cummings, J. H., and Macfarlane, G. T. (1991). The control and consequences of bacterial fermentation in the human colon. Journal of Applied Bacteriology, 70, 443-459.

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. E., and Macfarlane, G. T. (1987). Short chain fatty-acids in human large-intestine, portal, hepatic and venous-blood. Gut, 28, 1221-1227.

Dal Bello, F., Walter, J., Hammes, W. P., and Hertel, C. (2003). Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition. Microbial Ecology, 45, 455-463.

De Angelis, M., and Gobbetti, M. (2004b). Environmental stress responses in *Lactobacillus*: a review. Proteomics, 4, 106-122.

De Angelis, M., Di Cagno, R., Huet, C., Crecchio, C., Fox, P. F., and Gobbetti, M. (2004a). Heat shock response in *Lactobacillus plantarum*. Applied and Environmental Microbiology, 70, 1336-1346.

de Hoon, M. J. L., Imoto, S., Nolan, J., and Miyano, S. (2004). Open source clustering software. Bioinformatics, 20, 1453-1454.

de Leeuw, E., Li, X. Q., and Lu, W. Y. (2006). Binding characteristics of the *Lactobacillus brevis* ATCC 8287 surface layer to extracellular matrix proteins. FEMS Microbiology Letters, 260, 210-215.

De Preter, V., Hamer, H. M., Windey, K., and Verbeke, K. (2011). The impact of pre-and/or probiotics on human colonic metabolism: does it affect human health? Molecular Nutrition and Food Research, 55, 46-57.

Deepika, G., and D. Charalampopoulos. (2010). Surface and adhesion properties of lactobacilli. Advances in applied microbiology 70,127-152.

Deepika, G., Karunakaran, E., Hurley, C. R., Biggs, C. A., and Charalampopoulos, D. (2012). Influence of fermentation conditions on the surface properties and adhesion of *Lactobacillus rhamnosus* GG. Microbial Cell Factories, 11, 1-12.

delCardayre, S. B., and Davies, J. E. (1998). *Staphylococcus aureus* coenzyme A disulfide reductase, a new subfamily of pyridine nucleotide-disulfide oxidoreductase - sequence, expression, and analysis of *cdr*. Journal of Biological Chemistry, 273, 5752-5757.

Deplancke, B., and Gaskins, H. R. (2001). Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. American Journal of Clinical Nutrition, 73, 1131S-1141S.

Desmond, C., Fitzgerald, G. F., Stanton, C., and Ross, R. P. (2004). Improved stress tolerance of GroESL-overproducing Lactococcus lactis and orobiotic *Lactobacillus paracasei* NFBC 338. Applied and Environmental Microbiology, 70(10), 5929-5936. Desvaux, M., Dumas, E., Chafsey, I., and Hebraud, M. (2006). Protein cell surface display in Gram-positive bacteria: from single protein to macromolecular protein structure. FEMS Microbiology Letters, 256, 1-15.

Dhanani, A. S., and Bagchi, T. (2013). *Lactobacillus plantarum* CS24.2 prevents *Escherichia coli* adhesion to HT-29 cells and also down-regulates enteropathogen-induced tumor necrosis factor-α and interleukin-8 expression. Microbiology and Immunology, 57, 309-315.

Diancourt, L., Passet, V., Chervaux, C., Garault, P., Smokvina, T. and Brisse, S. (2007). Multilocus sequence typing of *Lactobacillus casei* reveals a clonal population structure with low levels of homologous recombination. Applied and Environmental Microbiology, 73, 6601-6611.

Diaz-Muniz, I., Banavara, D. S., Budinich, M. F., Rankin, S. A., Dudley, E. G., and Steele, J. L. (2006). *Lactobacillus casei* metabolic potential to utilize citrate as an energy source in ripening cheese: a bioinformatics approach. Journal of Applied Microbiology, 101, 872-882.

Douillard, F.P., Kant, R., Ritari, J., Paulin, L., Palva, A., de Vos, W.M. (2013). Comparative genome analysis of *Lactobacillus casei* strains isolated from Actimel and Yakult products

reveals marked similarities and points to a common origin. Microbial Biotechnology. 6:576-87.

Dudley, E. G., and Steele, J. L. (2005). Succinate production and citrate catabolism by Cheddar cheese nonstarter lactobacilli. Journal of Applied Microbiology, 98, 14-23.

Dunne, C., O'mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'halloran, S., Feeney, M., Flynn, S., Fitzgerald, G., Daly, C., Kiely, B., O 'Sullivan, G. C., Shanahan, F. and Collins, J. K. (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. American Journal of Clinical Nutrition, 73, 386S-392S.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E. and Relman, D. A. (2005). Diversity of the human intestinal microbial flora. Science, 308, 1635-1638.

Emanuelsson, O., Brunak, S., Von Heijne, G. and Nielsen, H. (2007). Locating proteins in the cell using TargetP, SignalP and related tools. Nature Protocols, 2, 953-971.

Engelhardt, H. (2007a). Are S-layers exoskeletons? The basic function of protein surface layers revisited. Journal of Structural Biology, 160, 115-124.

Engelhardt, H. (2007b). Mechanism of osmoprotection by archaeal S-layers: A theoretical study. Journal of Structural Biology, 160, 190-199.

Etzold, S., Kober, O. I., MacKenzie, D. A., Tailford, L. E., Gunning, A. P., Walshaw, J. and Juge, N. (2014). Structural basis for adaptation of lactobacilli to gastrointestinal mucus. Environmental Microbiology, 16, 888-903.

Fernandez, A., Ogawa, J., Penaud, S., Boudebbouze, S., Ehrlich, D., van de Guchte, M., and Maguin, E. (2008). Rerouting of pyruvate metabolism during acid adaptation in *Lactobacillus bulgaricus*. Proteomics, 8, 3154-3163.

Fernandez, M., and Zuniga, M. (2006). Amino acid catabolic pathways of lactic acid bacteria. Critical Reviews in Microbiology, 32, 155-183.

Fernández, M., Zúñiga, M. (2006). Amino acid catabolic pathways of lactic acid bacteria. Critical Reviews in Microbiology. 32:155–183.

Filannino, P., Cardinali, G., Rizzello, C., Buchin, S., De Angelis, M., Gobbetti, M., and Di Cagno, R. (2014). Metabolic responses of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices. Applied and Environmental Microbiology, 80, 2206-2215.

Fooks, L. J., and Gibson, G. R. (2002). Probiotics as modulators of the gut flora. British Journal of Nutrition, 88, S39-S49.

Fozo, E. M., Kajfasz, J. K., and Quivey, R. G. (2004). Low pH-induced membrane fatty acid alterations in oral bacteria. FEMS Microbiology Letters, 238, 291-295.

Francl, A. L., Hoeflinger, J. L., and Miller, M. J. (2012). Identification of lactose phosphotransferase systems in *Lactobacillus gasseri* ATCC 33323 required for lactose utilization. Microbiology-SGM, 158, 944-952.

Francl, A. L., Thongaram, T., and Miller, M. J. (2010). The PTS transporters of *Lactobacillus gasseri* ATCC 33323. BMC Microbiology, 10, 1-13.

Frece, J., Kos, B., Svetec, I. K., Zgaga, Z., Mrsa, V., and Suskovic, J. (2005). Importance of S-layer proteins in probiotic activity of *Lactobacillus acidophilus* M92. Journal of Applied Microbiology, 98, 285-292.

Frison-Norrie, S., and Sporns, P. (2001). Investigating the molecular heterogeneity of polysorbate emulsifiers by MALDI-TOF MS. Journal of Agricultural and Food Chemistry, 49, 3335-3340.

Fukushima, Y., Miyaguchi, S., Yamano, T., Kaburagi, T., Iino, H., Ushida, K., and Sato, K. (2007). Improvement of nutritional status and incidence of infection in hospitalised, enterally fed elderly by feeding of fermented milk containing probiotic *Lactobacillus johnsonii* La1 (NCC533). British Journal of Nutrition, 98, 969-977.

Gardiner, G. E., E. O'sullivan, J. Kelly, M. A. E. Auty, G. F. Fitzgerald, J. K. Collins, R. P. Ross, and C. Stanton. (2000). Comparative survival rates of human-derived Probiotic *Lactobacillus paracasei* and *Lactobacillus salivarius* strains during heat treatment and Spray drying. Applied and Environmental Microbiology 66, 2605-2612.

Gardiner, G., Stanton, C., Lynch, P. B., Collins, J. K., Fitzgerald, G., and Ross, R. P. (1999). Evaluation of Cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract. Journal of Dairy Science, 82, 1379–1387.

Garrote, G.L., Delfederico, L., Bibiloni, R., Abraham, A.G., Perez, P.F., Semorile, L., De Antoni, G.L. (2004) Lactobacilli isolated from kefir grains: evidence of the presence of S-layer proteins. Journal of Dairy Research. 71, 222–230

Gasser, F. (1970). Electrophoretic characterization of lactic dehydrogenases in the genus *Lactobacillus*. Journal of General Microbiology, 62, 223-239.

Gilman, J., and Cashman, K. D. (2006). The effect of probiotic bacteria on transepithelial calcium transport and calcium uptake in human intestinal-like Caco-2 cells. Current Issues in Intestinal Microbiology, 7, 1-6.

Gilmore, M. S., and Ferretti, J. J. (2003). Microbiology: The thin line between gut commensal and pathogen. Science, 299, 1999-2000.

Giraffa, G., Chanishvili, N., and Widyastuti, Y. (2010). Importance of lactobacilli in food and feed biotechnology. Research in Microbiology, 161, 480-487.

Glaasker, E., Tjan, F. S. B., Ter Steeg, P. F., Konings, W. N., and Poolman, B. (1998). Physiological response of *Lactobacillus plantarum* to salt and nonelectrolyte stress. Journal of Bacteriology, 180, 4718-4723.

Glenting, J., Beck, H. C., Vrang, A., Riemann, H., Ravn, P., Hansen, A. M. Madsen, S. (2013). Anchorless surface associated glycolytic enzymes from *Lactobacillus plantarum* 299v bind to epithelial cells and extracellular matrix proteins. Microbiological Research. 168, 245-253.

Gokce, E., Shuford, C. M., Franck, W. L., Dean, R. A., and Muddiman, D. C. (2011). Evaluation of Normalization Methods on GeLC-MS/MS Label-Free Spectral Counting Data to Correct for Variation during Proteomic Workflows. Journal of the American Society for Mass Spectrometry, 22, 2199-2208.

Gokce, E., Shuford, C.M., Franck, W.L., Dean, R.A., Muddiman, D.C. (2011). Evaluation of normalization methods on GeLC-MS/MS label-free spectral counting data to correct for variation during proteomic workflows. Journal of the American Society for Mass Spectrometry. 22, 2199−2208.

Golod, N. A., Loiko, N. G., Mulyukin, A. L., Neiymatov, A. L., Vorobjeva, L. I., Suzina, N. E., Shanenko, E. F., Gal'chenko, V. F. and El-Registan, G. I. (2009). Adaptation of lactic acid bacteria to unfavorable growth conditions. Microbiology, 78, 280-289.

Gopal, P. K., Prasad, J., Smart, J., and Gill, H. S. (2001). In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. International Journal Of Food Microbiology. 67, 207-216.

Granato, D., Perotti, F., Masserey, I., Rouvet, M., Golliard, M., Servin, A., and Brassart, D. (1999). Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. Applied and Environmental Microbiology, 65, 1071-1077.

Granato, D., Perotti, F., Masserey, I., Rouvet, M., Golliard, M., Servin, A., & Brassart, D. (1999). Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsoniiLa1* to human enterocyte-like Caco-2 cells. Applied and Environmental Microbiology. 65, 1071-1077.

Greene, J. D., and Klaenhammer, T. R. (1994). Factors involved in adherence of lactobacilli to human Caco-2 Cells. Applied and Environmental Microbiology, 60, 4487-4494.

Grosu-Tudor, S.-S., Zamfir, M., Van der Meulen, R., and De Vuyst, L. (2013). Isolation of novel homopolysaccharide-producing lactic acid bacteria from Romanian raw milk and fermented dairy products. European Food Research and Technology, 237, 609-615.

Guarner, F., and Malagelada, J.-R. (2003). Gut flora in health and disease. The Lancet, 361, 512-519.

Gyaneshwar, P., Paliy, O., McAuliffe, J., Jones, A., Jordan, M. I., and Kustu, S. (2005). Lessons from *Escherichia coli* genes similarly regulated in response to nitrogen and sulfur limitation. Proceedings of the National Academy of Sciences of the United States of America, 102, 3453-3458.

Hamon, E., Horvatovich, P., Marchioni, E., Aoudé-Werner, D., and Ennahar, S. (2014). Investigation of potential markers of acid resistance in *Lactobacillus plantarum* by comparative proteomics. Journal of Applied Microbiology, 116, 134-144.

Harper, D. S., and Loesche, W. J. (1984). Growth and bcid tolerance of human dental plaque bacteria. Archives of Oral Biology, 29, 843-848.

Hartke, A., Bouche, S., Gansel, X., Boutibonnes, P., and Auffray, Y. (1994). Starvation induced stress resistance in *Lactococcus lactis* subsp *lactis* IL1403. Applied and Environmental Microbiology, 60, 3474-3478.

Hartke, A., Giard, S.J.C., Benachour, A., Boutibonnes, P., Auffray, Y. (1996). The lactic acid stress response of Lactococcus lactis subsp. lactis. Current Microbiology. 33,194–199.

Hartl, F. U., and Hayer-Hartl, M. (2002). Protein folding - molecular chaperones in the cytosol: from nascent chain to folded protein. Science, 295, 1852-1858.

Hattori, M., and Taylor, T. D. (2009). The Human Intestinal Microbiome: A New Frontier of Human Biology. DNA Research, 16, 1-12.

Heilig, H., Zoetendal, E. G., Vaughan, E. E., Marteau, P., Akkermans, A. D. L., and de Vos, W. M. (2002). Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. Applied and Environmental Microbiology, 68, 114-123.

Helgeland, L., Vaage, J. T., Rolstad, B., Midtvedt, T., and Brandtzaeg, P. (1996). Microbial colonization influences composition and T-cell receptor VP repertoire of intraepithelial lymphocytes in rat intestine. Immunology, 89, 494-501.

Hemmerling, A., Harrison, W., Schroeder, A., Park, J., Korn, A., Shiboski, S. and Cohen, C. R. (2010). Phase 2a study assessing colonization efficiency, safety, and acceptability of Lactobacillus crispatus CTV-05 in women with bacterial vaginosis. Sexually Transmitted Diseases, 37, 745-750.

Hemmerling, A., Harrison, W., Schroeder, A., Park, J., Korn, A., Shiboski, S., & Cohen, C. R. (2009). Phase 1 dose-ranging safety trial of *Lactobacillus crispatus* CTV-05 (LACTIN-V) for the prevention of bacterial vaginosis. Sexually Transmitted Diseases, 36, 1-12.

Henderson, B. and Martin, A. (2011). Bacterial virulence in the moonlight: multitasking bacterial moonlighting proteins are virulence determinants in infectious disease. Infection and Immunity, 79, 3476-3491.

Hochwind, K., Weinmaier, T., Schmid, M., Van Hemert, S., Hartmann, A., Rattei, T., Rothballer, M. (2012). Draft genome sequence of *Lactobacillus* casei W56. Journal of Bacteriology, 194, 6638-6638.

Hou, J., Hannon, J. A., McSweeney, P. L. H., Beresford, T. P., and Guinee, T. P. (2012). Effect of curd washing on composition, lactose metabolism, pH, and the growth of non-starter lactic acid bacteria in full-fat Cheddar cheese. International Dairy Journal, 25, 21-25.

Howe, T. G. B., and Ward, J. M. (1975). The Utilization of Tween 80 as Carbon Source by *Pseudumonas*. Journal of General Microbiology, 92, 234-235

Huberts, D. H., and van der Klei, I. J. (2010). Moonlighting proteins: an intriguing mode of multitasking. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1803(4), 520-525.

Hüfner, E., Markieton, T., Chaillou, S., Crutz-Le Coq, A. M., Zagorec, M., and Hertel, C. (2007). Identification of *Lactobacillus sakei* genes induced during meat fermentation and their role in survival and growth. Applied and Environmental Microbiology, 73, 2522-2531.

Hurmalainen, V., Edelman, S., Antikainen, J., Baumann, M., Lähteenmäki, K. and Korhonen, T. K. (2007). Extracellular proteins of *Lactobacillus crispatus* enhance activation of human plasminogen. Microbiology, 153(4), 1112-1122.

Hussain, M. A., Knight, M. I., and Britz, M. L. (2009). Proteomic analysis of lactose-starved *Lactobacillus casei* during stationary growth phase. Journal of Applied Microbiology, 106, 764-773.

Hussain, M. A., Rouch, D. A., and Britz, M. L. (2009). Biochemistry of non-starter lactic acid bacteria isolate Lactobacillus casei GCRL163: Production of metabolites by stationary-phase cultures. International dairy journal, 19(1), 12-21.

Hutkins, R. W., and Nannen, N. L. (1993). pH Homeostasis in Lactic-Acid Bacteria. Journal of Dairy Science, 76(8), 2354-2365.

Hynonen, U., and Palva, A. (2013). *Lactobacillus* surface layer proteins: structure, function and applications. Applied Microbiology and Biotechnology, 97, 5225-5243.

Hynönen, U., Kant, R., Lähteinen, T., Pietilä, T. E., Beganovi, J., Smidt, H., Uroi, K., Åvall-Jääskeläinen, S. and Palva, A. (2014). Functional characterization of probiotic surface layer protein-carrying *Lactobacillus amylovorus* strains. BMC Microbiology, 14, 1-16.

Izquierdo, E., Horvatovich, P., Marchioni, E., Aoude-Werner, D., Sanz, Y., and Ennahar, S. (2009). 2-DE and MS analysis of key proteins in the adhesion of *Lactobacillus plantarum*, a first step toward early selection of probiotics based on bacterial biomarkers. Electrophoresis, 30, 949-956.

Jantzen, M., Göpel, A., and Beermann, C. (2013). Direct spray drying and microencapsulation of probiotic *Lactobacillus reuteri* from slurry fermentation with whey. Journal of Applied Microbiology, 115, 1029-1036.

Jardin, J., Mollé, D., Piot, M., Lortal, S., and Gagnaire, V. (2012). Quantitative proteomic analysis of bacterial enzymes released in cheese during ripening. International Journal of Food Microbiology, 155, 19–28

Jeffery, C. J. (1999). Moonlighting proteins. Trends in Biochemical Sciences. 24: 8–11.

Jensen, H., Roos, S., Jonsson, H., Rud, I., Grimmer, S., Van Pijkeren, J.-P., Britton, R. A. and Axelsson, L. (2014). The role of *Lactobacillus reuteri* cell and mucus binding protein A (CmbA) in adhesion to intestinal epithelial cells and mucus in vitro. Microbiology, 65, 671-681.

Joint, F., Agriculture, O., and World Health, O. (2006). Guidelines for the evaluation of probiotics in food. FAO Food and Nutrition, 85, 35-50.

Kainulainen, V. and Korhonen, T. K. 2014. Dancing to another tune—Adhesive moonlighting proteins in bacteria. Biology. 3, 178-204.

Kandler, O. (1983). Carbohydrate metabolism in lactic acid bacteria. Antonie Van Leeuwenhoek Journal of Microbiology, 49, 209-224.

Kashket, E. R. (1987). Bioenergetics of lactic-Acid bacteria - cytoplasmic pH and osmotolerance. FEMS Microbiology Reviews, 46, 233-244.

Kelly, D., and Conway, S. (2001). Genomics at work: the global gene response to enteric bacteria. Gut, 49, 612-613.

Kennedy, N. A., Walker, A. W., Berry, S. H., Duncan, S. H., Farquarson, F. M., Louis, P., Thomson, J. M., Satsangi, J., Flint, H. J., Parkhill, J., Lees, C. W., Hold, G. L. other members

not named within the manuscript author(2014). The impact of different DNA extraction kits and laboratories upon the assessment of human gut microbiota composition by 16S rRNA gene sequencing. PLoS ONE, 9, e88982.

Kerwin, B. A. (2008). Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. Journal of Pharmaceutical Sciences, 97, 2924-2935.

Kieronczyk, A., Skeie, S., Olsen, K., and Langsrud, T. (2001). Metabolism of amino acids by resting cells of non-starter lactobacilli in relation to flavour development in cheese. International Dairy Journal, 11, 217-224.

Kinoshita, H., Wakahara, N., Watanabe. (2008). Cell surface glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *Lactobacillus plantarum* LA 318 recognizes human A and B blood group antigens. Research in Microbiology, 159, 685–691.

Klaenhammer, T. R., Barrangou, R., Buck, B. L., Azcarate-Peril, M. A., and Altermann, E. (2005). Genomic features of lactic acid bacteria effecting bioprocessing and health. FEMS Microbiology Reviews, 29, 393-409.

Kleerebezem, M., and Vaughan, E. E. (2009). Probiotic and gut lactobacilli and bifidobacteria: molecular approaches to study diversity and activity. Annual Review of Microbiology, 63, 269-290.

Klumpp, S., Zhang, Z. G., and Hwa, T. (2009). Growth rate-dependent global effects on gene expression in bacteria. Cell, 139, 1366-1375.

Knight, D. J. W., and Girling, K. J. (2003). Gut flora in health and disease. Lancet, 361, 1831-1831.

Kocharunchitt, C., King, T., Gobius, K., Bowman, J. P., and Ross, T. (2012). Integrated transcriptomic and proteomic analysis of the physiological response of *Escherichia coli* O157:H7 Sakai to steady-state conditions of cold and water activity stress. Molecular and Cellular Proteomics, 11, 1-18.

Koponen, J., Laakso, K., Koskenniemi, K., Kankainen, M., Savijoki, K., Nyman, T. A., De Vos, W. M., Tynkkynen, S., Kalkkinen, N. and Varmanen, P. Varmanen, P. (2012). Effect of acid stress on protein expression and phosphorylation in *Lactobacillus rhamnosus* GG. Journal of Proteomics, 75, 1357-1374. Kos, B., Suskovic, J., Vukovic, S., Simpraga, M., Frece, J., and Matosic, S. (2003). Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. Journal of Applied Microbiology, 94, 981-987.

Larsen, N., Nissen, P., and Willats, W. G. T. (2007). The effect of calcium ions on adhesion and competitive exclusion of *Lactobacillus* ssp and *E. coli* O138. International Journal of Food Microbiology, 114, 113-119.

Lavermicocca, P., Valerio, F., Lonigro, S. L., De Angelis, M., Morelli, L., Callegari, M. L., Rizzello, C. G. and Visconti, A. (2005). Study of adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotic food. Applied and Environmental Microbiology, 71, 4233-4240.

Law, B. A. (2001). Controlled and accelerated cheese ripening: the research base for new technologies. International Dairy Journal, 11, 383-398.

Lebeer, S., Vanderleyden, J., and De Keersmaecker, S. C. J. (2008). Genes and molecules of lactobacilli supporting probiotic action. Microbiology and Molecular Biology Reviews, 72, 728-764.

Lee, K., Lee, H. G., Pi, K., and Choi, Y. J. (2008). Effect of low pH on protein expression by the probiotic bacterium *Lactobacillus reuteri*. Proteomics, 8, 1624-1630.

Lepage, P., Leclerc, M. C., Joossens, M., Mondot, S., Blottiere, H. M., Raes, J., Ehrlich, D. and Dore, J. (2013). A metagenomic insight into our gut's microbiome. Gut, 62, 146-158.

Leroy, F., and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science and Technology, 15, 67-78.

Ley, R. E. (2014). Harnessing Microbiota to Kill a Pathogen: The sweet tooth of *Clostridium difficile*. Nature medicine, 20, 248-249.

Li, C. Y., Cheng, C. Y., and Chen, T. L. (2004). Fed-batch production of lipase by *Acinetobacter radioresistens* using Tween 80 as the carbon source. Biochemical Engineering Journal, 19, 25-31.

Li, Qing, Xiaoli Liu12, Mingsheng Dong, Jianzhong Zhou, and Ying Wang. (2015). Adhesion and probiotic properties of *Lactobacillus plantarum* isolated from Chinese traditional fermented soybean paste. Global Advanced Research Journal of Food Science and Technology, 4, 1-9.

Liu, H. B., Sadygov, R. G., and Yates, J. R. (2004). A model for random sampling and estimation of relative protein abundance in shotgun proteomics. Analytical Chemistry, 76, 4193-4201.

Liu, H., Dicksved, J., Lundh, T., and Lindberg, J. E. (2014). Heat shock proteins: intestinal gatekeepers that are influenced by dietary components and the gut microbiota. Pathogens, 3, 187-210.

Lorca, G. L., Wadstrom, T., de Valdez, G. F., and Ljungh, A. (2001). *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori* in vitro. Current Microbiology, 42, 39-44.

Lorca, G., Torino, M. I., de Valdez, G. F., and Ljungh, A. (2002). Lactobacilli express cell surface proteins which mediate binding of immobilized collagen and fibronectin. FEMS Microbiology Letters, 206, 31-37.

Lortal, S., Vanheijenoort, J., Gruber, K., and Sleytr, U. B. (1992). S-Layer of *Lactobacillus helveticus* ATCC-12046 - isolation, chemical characterization and re-formation after extraction with lithium chloride. Journal of General Microbiology, 138, 611-618.

Ltermann, E., Russell, W. M., Azcarate-Peril, M. A., Barrangou, R., Buck, B. L., Mcauliffe, O., Souther, N., Dobson, A., Duong, T., Callanan, M., Lick, S., Hamrick, A., Cano, R. and Klaenhammer, T. R. (2005). Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. Proceedings of the National Academy of Sciences. 102, 3906-3912.

Mack, D., Ahrné, S., Hyde, L., Wei, S. and Hollingsworth, M. (2003). Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. Gut, 52, 827-833.

MacKenzie, D. A., Faye J., Mary L. P. R, Amandine V., Roy J. B., Stefan R., Jens W. and Nathalie J. Strain-specific diversity of mucus-binding proteins in the adhesion and aggregation properties of *Lactobacillus reuteri*. Microbiology, 156, (2010): 3368-3378.

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A., Pavlova, N., Karamychev, V., Polouchine, N., Shakhova, V., Grigoriev, I., Lou, Y., Rohksar, D., Lucas, S., Huang, K., Goodstein, D.M., Hawkins, T., Plengvidhya, V., Welker, D., Hughes, J., Goh, Y., Benson, A., Baldwin, K., Lee, J.H., Diaz-Muniz, I., Dosti, B., Smeianov, V., Wechter, W., Barabote, R., Lorca, G., Altermann, E., Barrangou, R., Ganesan, B., Xie, Y., Rawsthorne, H., Tamir, D., Parker, C., Breidt, F., Broadbent, J., Hutkins, R., O'sullivan, D., Steele, J., Unlu, G., Saier, M., Klaenhammer, T., Richardson, P., Kozyavkin, S,, Weimer, B., Mills, D. (2006). Comparative genomics of the Lactic Acid Bacteria. Proceedings of the National Academy of Sciences. 103, 15611-15616.

Mangiapane, E., Lamberti, C., Pessione, A., Ceruti, P., Novelli, F., Galano, E., Virkola, R., Korhonen, T. and Pessione, E. Pessione, E. (2013). An integrated proteomic and physiological approach to understand the adhesion mechanism of the probiotic *Lactobacillus reuteri* Lb2 BM DSM 16143. Journal of Integrated OMICS, 3, 145-156.

Maqueda, M., Sanchez-Hidalgo, M., Fernandez, M., Montalban-Lopez, M., Valdivia, E., and Martinez-Bueno, M. (2008). Genetic features of circular bacteriocins produced by Gram-positive bacteria. FEMS Microbiology Reviews, 32, 2-22.

Martinez, B., Sillanpaa, J., Smit, E., Korhonen, T. K., and Pouwels, P. H. (2000). Expression of cbsA encoding the collagen-binding S-protein of *Lactobacillus crispatus* JCM5810 in *Lactobacillus casei* ATCC 393(T). Journal of Bacteriology, 182, 6857-6861. Martley, F., and Crow, V. (1993). Interactions between non-starter microorganisms during cheese manufacture and repening. International Dairy Journal, 3, 461-483.

Masuda, K., and Kawata, T. (1981). Characterization of a regular array in the wall of *Lactobacillus buchneri* and its reattachment to the other wall components. Journal of general microbiology, 124, 81-90.

Maze, A., Boel, G., Zuniga, M., Bourand, A., Loux, V., Yebra, M. J., Monedero, V., Correia, K., Jacques, N., Beaufils, S., Poncet, S., Joyet, P., Milohanic, E., Casaregola, S., Auffray, Y., Perez-Martinez, G., Gibrat, J. F., Zagorec, M., Francke, C., Hartke, A. and Deutscher, J. (2010). Complete genome sequence of the probiotic *Lactobacillus casei* Strain BL23. Journal of Bacteriology, 192, 2647-2648. Mazmanian, S. K., and Lee, Y. K. (2014). Interplay between intestinal microbiota and host immune system. Journal of Bacteriology and Virology, 44, 1-9.

McCartney, A. L. and Gibson, G. R. (2006). The normal microbiota of the human gastrointestinal tract: history of analysis, succession, and dietary influences. Gastrointestinal Microbiology, 1, 51-73.

McCracken, V. J., and Lorenz, R. G. (2001). The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. Cellular Microbiology, 3, 1-11.

Mcsweeney, P., Walsh, E., Fox, P., Cogan, T., Drinan, F. and Castelo-Gonzalez, M. (1994). A procedure for the manufacture of Cheddar cheese under controlled bacteriological conditions and the effect of adjunct lactobacilli on cheese quality. Irish Journal of Agricultural and Food Research, 33, 183-192.

Mills, S., Stanton, C., Fitzgerald, G. F., and Ross, R. P. (2011). Enhancing the stress responses of probiotics for a lifestyle from gut to product and back again. Microbial Cell Factorie, 10, 1-15.

Millsap, K. W., Reid, G., Mei, H. C. v. d., and Busscher, H. J. (1997). Cluster analysis of genotypically characterized *Lactobacillus* species based on physicochemical cell surface properties and their relationship with adhesion to hexadecane. Canadian Journal of Microbiology, 43, 284-291.

Minervini, F., Siragusa, S., Faccia, M., Dal Bello, F., Gobbetti, M., and De Angelis, M. (2012). Manufacture of Fior di Latte cheese by incorporation of probiotic lactobacilli. Journal of Dairy Science, 95, 508-520.

Munns, R., Greenway, H., Setter, T. L., and Kuo, J. (1983). Turgor pressure, volumetric elastic-modulus, osmotic volume and ultrastructure of *Chlorella emersonii* grown at high and low external NaCl. Journal of Experimental Botany, 34, 144-155.

Muñoz-Provencio, Diego, Marta Llopis, María Antolín, Inés De Torres, Francisco Guarner, Gaspar Pérez-Martínez, and Vicente Monedero. (2009) .Adhesion properties of *Lactobacillus casei* strains to resected intestinal fragments and components of the extracellular matrix. Archives of Microbiology, 191, 153-161.

Nakamura, C. E., and Whited, G. M. (2003). Metabolic engineering for the microbial production of 1, 3-propanediol. Current Opinion in Biotechnology, 14, 454-459.

Nannen, N. L., and Hutkins, R. W. (1991). Intracellular pH effects in lactic-acid bacteria. Journal of Dairy Science, 74, 741-746.

Neish, A. S. (2009). Microbes in gastrointestinal health and disease. Gastroenterology, 136, 65-80.

Nesvizhskii, A. I., Keller, A., Kolker, E., and Aebersold, R. (2003). A statistical model for identifying proteins by tandem mass spectrometry. Analytical Chemistry, 75, 4646-4658.

Nesvizhskii, A.I., Keller, A., Kolker, E. Aebersold, R. (2003). A statistical model for identifying proteins by tandem mass spectrometry. Analytical Chemistry, 75, 4646-4658.

Nezhad, M. H., Hussain, M. A., and Britz, M. L. (2015). Stress responses in probiotic *Lactobacillus casei*. Critical Reviews in Food Science and Nutrition, 55, 740-749.

Nezhad, M. H., Knight, M., and Britz, M. L. (2012). Evidence of changes incell surface proteins during growth of *Lactobacillus casei* under cidic conditions. Food Science and Biotechnology, 21, 253-260.

Nichols, D. S. and Mcmeekin, T. A. (2002). Biomarker techniques to screen for bacteria that produce polyunsaturated fatty acids. Journal of Microbiological Methods, 48, 161-170.

Nishiyama, Keita, Koichi Nakamata, Shintaro Ueno, Akari Terao, Ni Putu Desy Aryantini, I. Nengah Sujaya, Kenji Fukuda, Tadasu Urashima, Yuji Yamamoto, and Takao Mukai.

(2014). Adhesion properties of *Lactobacillus rhamnosus* mucus-binding factor to mucin and extracellular matrix proteins. Bioscience, Biotechnology, and Biochemistry. 79, 271-279.

Nivoliez, Adrien, Philippe Veisseire, Elina Alaterre, Caroline Dausset, Fabrice Baptiste, Olivier Camarès, Marylise Paquet-Gachinat, Muriel Bonnet, Christiane Forestier, and Stéphanie Bornes. (2015). Influence of manufacturing processes on cell surface properties of probiotic strain *Lactobacillus rhamnosus* Lcr35. Applied Microbiology and Biotechnology, 99, 399-411.

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions–a review. International Journal of Medical Microbiology, 300, 57-62.

O'Flaherty, S., and Klaenhammer, T. R. (2010). The role and potential of probiotic bacteria in the gut, and the communication between gut microflora and gut/host. International Dairy Journal. 20, 262-268.

Oh, S.; Rheem, S.; Sim, J.; Kim, S.; Baek, Y. (1995). Optimizing conditions for the growth of *Lactobacillus casei* YIT 9018 in tryptone-yeast extract glucose medium by using response-surface methodology. Applied and Environmental Microbiology, 61, 3809−3814.

Old, W. M., Meyer-Arendt, K., Aveline-Wolf, L., Pierce, K. G., Mendoza, A., Sevinsky, J. R., Katheryn A. R., Ahn, N. G. (2005). Comparison of label-free methods for quantifying human proteins by shotgun proteomics. Molecular and Cellular Proteomics. 4, 1487-1502.

Olson, N. F. The impact of lactic acid bacteria on cheese flavor. (1990). FEMS Microbiology Reviews, 87, 131−47.

Orrhage, K., and Nord, C. (1999). Bifidobacteria and lactobacilli in human health. Drugs under Experimental and Clinical Research, 26, 95-111.

Ouwehand, A. C., Kirjavainen, P. V., Gronlund, M. M., Isolauri, E., and Salminen, S. J. (1999). Adhesion of probiotic micro-organisms to intestinal mucus. International Dairy Journal, 9, 623-630.

Ouwehand, A. C., Tuomola, E. M., Tolkko, S., and Salminen, S. (2001). Assessment of adhesion properties of novel probiotic strains to human intestinal mucus. International Journal of Food Microbiology, 64, 119-126.

Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A., and Brown, P. O. (2007). Development of the human infant intestinal microbiota. Plos Biology, 5, 1556-1573.

Palomar, M. M., Maldonado Galdeano, C., and Perdigón, G. (2014). Influence of a probiotic *Lactobacillus* strain on the intestinal ecosystem in a stress model mouse. Brain, Behavior, and Immunity, 35, 77-85.

Partanen, L., Marttinen, N. and Alatossava, T. (2001). Fats and fatty acids as growth factors for *Lactobacillus delbrueckii*. Systematic and Applied Microbiology, 24, 500-506.

Parvez, S., Malik, K. A., Kang, S. A., and Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology, 100, 1171-1185.

Paul, S. C., Jain, P., Mitra, J., Dutta, S., Bhattacharya, P., Bal, B., Bhattacharyya, D., Das Gupta, S. and Pal, S. (2012). Induction of Cr(VI) reduction activity in an *Anoxybacillus* strain under heat stress: a biochemical and proteomic study. FEMS Microbiology Letters, 331, 70-80.

Peters, J., Nitsch, M., Kuhlmorgen, B., Golbik, R., Lupas, A., Kellermann, J., Engelhardt, H., Pfander, J. P., Muller, S., Goldie, K., Engel, A., Stetter, K. O. and Baumeister, W. (1995). Tetrabrachion A Filamentous Archaebacterial Surface Protein Assembly of Unusual Structure and Extreme Stability. Journal of Molecular Biology, 245, 385-401.

Poolman, B. (2002). Transporters and their roles in LAB cell physiology. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 82, 147-164.

Poolman, B., and Glaasker, E. (1998). Regulation of compatible solute accumulation in bacteria. Molecular Microbiology. 29, 397-407.

Poolman, B., Molenaar, D., Smid, E.J., Ubbink, T., Abee, T., Renault, P.P., Konings, W.N. (1991). Malolactic fermentation: electrogenic malate uptake and malate/lactate antiport generate metabolic energy. Journal of Bacteriology, 173, 6030–6037.

Poppinga, L., Janesch, B., Fuenfhaus, A., Sekot, G., Garcia-Gonzalez, E., Hertlein, G., Hedtke, K., Schaeffer, C. and Genersch, E. (2012). Identification and functional analysis of the S-layer protein SplA of *Paenibacillus larvae*, the causative agent of American Foulbrood of honey bees. Plos Pathogens, 8, 1-13.

Prasad, J., McJarrow, P., and Gopal, P. (2003). Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. Applied and Environmental Microbiology, 69, 917-925.

Puertollano, E., Puertollano, M. A., Cruz-Chamorro, L., de Cienfuegos, G. A., Ruiz-Bravo, A., and de Pablo, M. A. (2008). Orally administered *Lactobacillus plantarum* reduces pro-inflammatory interleukin secretion in sera from *Listeria monocytogenes* infected mice. British Journal of Nutrition, 99, 819-825.

Qin, H. L., Zhang, Z. W., Hang, X. M., and Jiang, Y. Q. (2009). *Lactobacillus plantarum* prevents Enteroinvasive *Escherichia coli* induced tight junction proteins changes in intestinal epithelial cells. BMC Microbiology, 9, 1-9.

Quehenberger, O. and Dennis, E. A. (2011). The human plasma lipidome. New England Journal of Medicine, 365, 1812-1823.

Quigley, E. M. M. (2010). Prebiotics and probiotics; modifying and mining the microbiota. Pharmacological Research, 61, 213-218.

Quintans, N. G., Blancato, V., Repizo, G., Magni, C., and Lopez, P. (2008). Citrate metabolism and aroma compound production in lactic acid bacteria. 37, 66-88.

Quivey, J.R.R.G., Faustoferri, R.C., Clancy, K.A., Marquis, R.E. (1995). Acid adaptation in *Streptococcus mutans* UA159 alleviates sensitization to environmental stress due to RecA deficiency. FEMS Microbiology Letters, 126, 257–261.

Rajilić-Stojanović, M., and Vos, W. M. (2014). The first 1,000 cultured species of the human gastrointestinal microbiota. FEMS Microbiology Reviews, 38, 996-1047

Ramiah, K., van Reenen, C.A., Dicks, L.M.T. (2008). Surface-bound proteins of *Lactobacillus plantarum* 423 that contribute to adhesion of Caco-2 cells and their role in competitive exclusion and displacement of *Clostridium sporogenes* and *Enterococcus faecalis*. Research in Microbiology, 159, 470–475.

Rauch, A., Bellew, M., Eng, J., Fitzgibbon, M., Holzman, T., Hussey, P., Igra, M., Maclean, B., Lin, C. W., Detter, A., Fang, R. H., Faca, V., Gafken, P., Zhang, H., Whiteaker, J., States, D., Hanash, S., Paulovich, A. and Mcintosh, M. W. (2006). Computational proteomics analysis system (CPAS): an extensible, open-source analytic system for evaluating and publishing proteomic data and high throughput biological experiments. Journal of Proteome Research, 5, 1024-1024.

Ravcheev, D. A., Best, A. A., Sernova, N. V., Kazanov, M. D., Novichkov, P. S., and Rodionov, D. A. (2013). Genomic reconstruction of transcriptional regulatory networks in lactic acid bacteria. BMC Genomics, 14, 94.

Rehn, U., Vogensen, F. K., Persson, S. E., Saeden, K. H., Nilsson, B. F., and Ardo, Y. (2011). Influence of microflora on texture and contents of amino acids, organic acids, and volatiles in semi-hard cheese made with DL-starter and propionibacteria. Journal of Dairy Science, 94, 1098-1111.

Reid, G. (1999). The scientific basis for probiotic strains of *Lactobacillus*. Applied and Environmental Microbiology, 65, 3763-3766.

Renault, P., Gaillardin, C., Heslot, H. 1988. Role of malolactic fermentation in lactic acid bacteria. Biochimie, 70, 375–379.

Revilla-Guarinos, A., Alcántara, C., Rozès, N., Voigt, B., and Zúñiga, M. (2014). Characterization of the response to low pH of *Lactobacillus casei* ΔRR12, a mutant strain with low D-alanylation activity and sensitivity to low pH. Journal of Applied Microbiology, 116, 1250-1261.

Roberfroid, M. B., Bornet, F., Bouley, C., and Cummings, J. H. (1995). Colonic microflora - nutrition and health - summary and conclusions of an International Life Sciences Institute (Ilsi) Europe Workshop held in Barcelona, Spain. Nutrition Reviews, 53, 127-130.

Rojas, M., Ascencio, F., and Conway, P. L. (2002). Purification and characterization of a surface protein from *Lactobacillus fermentum* 104R that binds to porcine small intestinal mucus and gastric mucin. Applied and Environmental Microbiology, 68, 2330-2336.

Roos, S., and Jonsson, H. (2002). A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. Microbiology-SGM, 148, 433-442.

Rubio, R., Jofré, A., Aymerich, T., Guàrdia, M. D., and Garriga, M. (2014). Nutritionally enhanced fermented sausages as a vehicle for potential probiotic lactobacilli delivery. Meat Science, 96, 937-942.

Rul, F., Zagorec, M., and Champomier-Vergès, M.-C. (2013). Lactic acid bacteria in fermented foods. Proteomics in Foods (pp. 261-283): Springer.

Russell, J. B., and DiezGonzalez, F. (1998). The effects of fermentation acids on bacterial growth. In R. K. Poole (Ed.), Advances in Microbial Physiology, Vol 39, 205-234.

Saarela, M., Mogensen, G., Fonden, R., Matto, J., and Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology, 84, 197-215.

Saarela, M., Rantala, M., Hallamaa, K., Nohynek, L., Virkajarvi, I., and Matto, J. (2004). Stationary-phase acid and heat treatments for improvement of the viability of probiotic lactobacilli and bifidobacteria. Journal of Applied Microbiology, 96, 1205-1214.

Saldanha, A. J. (2004). Java Treeview-extensible visualization of microarray data. Bioinformatics, 20, 3246-3248.

Sanchez, B., Bressollier, P. and Urdaci, M. C. (2008). Exported proteins in probiotic bacteria: adhesion to intestinal surfaces, host immunomodulation and molecular cross-talking with the host. FEMS Immunology and Medical Microbiology, 54, 1-17.

Sanchez, B., Bressollier, P., Chaignepain, S., Schmitter, J. M., and Urdaci, M. C. (2009). Identification of surface-associated proteins in the probiotic bacterium *Lactobacillus rhamnosus* GG. International Dairy Journal, 19, 85-88.

Sanders, M. E., Akkermans, L. M. A., Dirk Hall Schar-Zammaretti er, C. H., Heimbach, J., Hörmannsperger, G., Geert Huys, D. D. L. and Vaughan, E. (2010). Safety assessment of probiotics for human use. Gut Microbes, 3, 164-185.

Saxelin, M., Rautelin, H., Salminen, S., Mäkelä, P.H. (1996). Safety of commercial products with viable *Lactobacillus* strains. Infectious Diseases in Clinical Practice, 5, 331-335.

Schaer-Zammaretti, P., and Ubbink, J. (2003). Imaging of lactic acid bacteria with AFM—elasticity and adhesion maps and their relationship to biological and structural data. Ultramicroscopy, 97, 199-208.

Schar-Zammaretti, P. and Ubbink, J. (2003). The cell wall of lactic acid bacteria: Surface constituents and macromolecular conformations. Biophysical Journal. 85, 4076-4092.

Schär-Zammaretti, P., and Ubbink, J. (2003). The cell wall of lactic acid bacteria: surface constituents and macromolecular conformations. Biophysical Journal, 85, 4076-4092.

Schneitz, C., Nuotio, L., and Lounatma, K. (1993). Adhesion of *Lactobacillus acidophilus* to avian intestinal epithelial cells mediated by the crystalline bacterial-Cell Surface-Layer (S-Layer). Journal of Applied Bacteriology, 74, 290-294.

Searle, B. C. (2010). Scaffold: A bioinformatic tool for validating MS/MS-based proteomic studies. Proteomics, 10, 1265-1269.

Sebald, W., Friedl, P., Schairer, H. U., and Hoppe, J. (1982). Structure and Genetics of The H+-Conducting Portion of the Atp Synthase. Annals of the New York Academy of Sciences, 402, 28-44.

Sedewitz, B., Schleifer, K. H., and Gotz, F. (1984). Physiological role of pyruvate oxidase in the aerobic metabolism of *Lactobacillus plantarum*. Journal of Bacteriology, 160, 462-465.

Selle, K., and Klaenhammer, T. R. (2013). Genomic and phenotypic evidence for probiotic influences of *Lactobacillus gasseri* on human health. FEMS Microbiology Reviews, 37, 915-935.

Servin, A. L. (2003). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiology Reviews, 28, 405-440.

Servin, A. L., and Coconnier, M. H. (2003). Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. Best Practice and Research in Clinical Gastroenterology, 17, 741-754.

Sghir, A., Gramet, G., Suau, A., Rochet, V., Pochart, P., and Dore, J. (2000). Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. Applied and Environmental Microbiology, 66, 2263-2266.

Shabala, L., Mcmeekin, T., Budde, B. B. and Siegumfeldt, H. (2006). *Listeria innocua* and *Lactobacillus delbrueckii* subsp. *bulgaricus* employ different strategies to cope with acid stress. International Journal of Food Microbiology, 110, 1-7.

Shanahan, F. (2002). The host-microbe interface within the gut. Best Practice and Research in Clinical Gastroenterology, 16, 915-931.

Shao, L., Wu, Z., Zhang, H., Chen, W., Ai, L., and Guo, B. (2014). Partial characterization and immunostimulatory activity of exopolysaccharides from *Lactobacillus rhamnosus* KF5. Carbohydrate Polymers, 107, 51-56.

Sharma, M., and Devi, M. (2014). Probiotics: a comprehensive approach toward health foods. Critical Reviews in Food Science and Nutrition, 54, 537-552.

Sidhu, P., Sharma, R., Soni, S. K., and Gupta, J. K. (1998). Production of extracellular alkaline lipase by a new thermophilic *Bacillus* sp. Folia Microbiologica, 43, 51-54.

Sidira, M., Galanis, A., Nikolaou, A., Kanellaki, M., and Kourkoutas, Y. (2014). Evaluation of *Lactobacillus casei* ATCC 393 protective effect against spoilage of probiotic dry-fermented sausages. Food Control, 42, 315-320.

Sirover, M. A. (2012). Subcellular dynamics of multifunctional protein regulation: mechanisms of GAPDH intracellular translocation. Journal of Cellular Biochemistry, 113, 2193-2200.

Sleytr, U. B. (1978). Regular arrays of macromolecules on bacterial cell walls: structure, chemistry, assembly, and function. International Review of Cytology, 53, 1-64.

Sleytr, U. B., Messner, P., Pum, D., and Sára, M. (1993). Crystalline bacterial cell surface layers. Molecular microbiology, 10, 911-916.

Smeds, A., Varmanen, P., and Palva, A. (1998). Molecular characterization of a stress-inducible gene from *Lactobacillus helveticus*. Journal of Bacteriology, 180, 6148-6153.

Smit, E., Oling, F., Demel, R., Martinez, B., and Pouwels, P. H. (2001). The S-layer Protein of *Lactobacillus acidophilus* ATCC 4356: Identification and Characterisation of Domains Responsible for S-protein Assembly and Cell Wall Binding. Journal of Molecular Biology, 305, 245-257.

Spano, G., Beneduce, L., Perrotta, C., Massa, S., (2005). Cloning and characterization of the hsp 18.55 gene, a new member of the small heat shock genes family isolated from wine Lactobacillus plantarum. Research in Microbiology, 156, 219e224.

Starrenburg, M. J. C., and Hugenholtz, J. (1991). Citrate Fermentation By *Lactococcus* and *Leuconostoc* Spp. Applied and Environmental Microbiology, 57, 3535-3540.

Steele, J. L. (2006). Conditions required for citrate utilization during growth of *Lactobacillus casei* ATCC334 in chemically defined medium and Cheddar cheese extract. Antonie van Leeuwenhoek, 90, 233-243.

Steele, J. L., Budinich, M. F., Cai, H., Curtis, S. C., and Broadbent, J. R. (2006). Diversity and metabolic activity of *Lactobacillus casei* in ripening cheddar cheese. Australian Journal of Dairy Technology, 61, 53-60.

Steensels, J., and Verstrepen, K. J. (2014). Taming Wild Yeast: Potential of Conventional and Nonconventional Yeasts in Industrial Fermentations. Annual Review of Microbiology, 68, 61-80.

Steer, T., Carpenter, H., Tuohy, K., and Gibson, G. R. (2000). Perspectives on the role of the human gut microbiota and its modulation by pro- and prebiotics. Nutrition Research Reviews, 13, 229-254.

Sulaiman, A. Q., Jalilian, F. A., Mustafa, S., Sekawi, Z., Ghazali, H. M., Hussin, A. S. M., and Yazid, A. M. (2009). Cholic acid resistance and the adherence ability of *Bifidobacterium pseudocaenulatum* G4. African Journal of Biotechnology, 8, 5066-5070.

Taguchi, H., Takahashi, M., Yamaguchi, H., Osaki, T., Komatsu, A., Fujioka, Y., and Kamiya, S. (2002). Experimental infection of germ-free mice with hyper-toxigenic enterohaemorrhagic *Escherichia coli* O157:H7, strain 6. Journal of Medical Microbiology, 51, 336-343.

Tan, W., Budinich, M., Ward, R., Broadbent, J. R. and Steele, J. (2012). Optimal growth of *Lactobacillus casei* in a Cheddar cheese ripening model system requires exogenous fatty acids. Journal of Dairy Science, 95, 1680-1689.

Tannock, G. W. (1999). Analysis of the intestinal microflora: a renaissance. Antonie Van Leeuwenhoek, 76, 265-278.

Tannock, G. W. (2004). A special fondness for lactobacilli. Applied and Environmental Microbiology, 70, 3189-3194.

Tannock, G. W. (2005). Probiotics and prebiotics: scientific aspects: Horizon Scientific Press.

Tannock, G. W., Munro, K., Harmsen, H. J. M., Welling, G. W., Smart, J., and Gopal, P. K. (2000). Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. Applied and Environmental Microbiology, 66, 2578-2588.

Taverniti, V., Stuknyte, M., Minuzzo, M., Arioli, S., De Noni, I., Scabiosi, C., Cordova, Z. M., Junttila, I., Hamalainen, S., Turpeinen, H., Mora, D., Karp, M., Pesu, M. and Guglielmetti, S. (2013). S-Layer Protein mediates the stimulatory effect of *Lactobacillus helveticus* MIMLh5 on innate immunity. Applied and Environmental Microbiology, 79, 1221-1231.

Teixeira, P., Castro, H., MohacsiFarkas, C., and Kirby, R. (1997). Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress. Journal of Applied Microbiology, 83, 219-226.

Thomas, T. D. (1986). Oxidative Activity of Bacteria from Cheddar Cheese. New Zealand Journal of Dairy Science and Technology, 21, 37-47.

Tiittanen, M., Keto, J., Haiko, J., Mättö, J., Partanen, J., and Lähteenmäki, K. (2013). Interaction with intestinal epithelial cells promotes an immunosuppressive phenotype in *Lactobacillus casei*. PLoS ONE, 8, e78420.

Titgemeyer, F., and Hillen, W. (2002). Global control of sugar metabolism: a Gram-positive solution. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 82, 59-71.

Tjalsma, H., Lambooy, L., Hermans, P. W., and Swinkels, D. W. (2008). Shedding and shaving: disclosure of proteomic expressions on a bacterial face. Proteomics, 8, 1415-1428.

Tomaro-Duchesneau, C., Saha, S., and Prakash, S. (2014). Modification of the gut microbiota to promote human health. Probiotics, Prebiotics and Gut Health, 15, 15-34.

Tomita, H., Kamei, E., and Ike, Y. (2008). Cloning and genetic analyses of the bacteriocin 41 determinant encoded on the *Enterococcus faecalis* pheromone-responsive conjugative plasmid pYI14: a novel bacteriocin complemented by two extracellular components (lysin and activator). Journal of Bacteriology, 190, 2075-2085.

Tsakalidou, E.; Papadimitriou, K. (2011). Stress responses of lactic acid bacteria,Responses of Lactic Acid Bacteria to Starvation; Springer Publishing: New York, 199−218.

Tuohy, K. M., Probert, H. M., Smejkal, C. W., and Gibson, G. R. (2003). Using probiotics and prebiotics to improve gut health. Drug Discovery Today, 8, 692-700.

Tuomola, E. M., and Salminen, S. J. (1998). Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures. International Journal of Food Microbiology, 41, 45-51.

Tuomola, E. M., Ouwehand, A. C., and Salminen, S. J. (2000). Chemical, physical and enzymatic pre-treatments of probiotic lactobacilli alter their adhesion to human intestinal mucus glycoproteins. International Journal of Food Microbiology, 60, 75-81.

Tuomola, Elina M., and Seppo J. Salminen. (1998). Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures. International Journal of Food Microbiology 41, 45-51.

Turpin, W., Humblot, C., Thomas, M., and Guyot, J.-P. (2010). Lactobacilli as multifaceted probiotics with poorly disclosed molecular mechanisms. International Journal of Food Microbiology, 143, 87-102.

Ur, S. R.; Waldron, D.; Fox, P. F. (2004). Effect of modifying lactose concentration in cheese curd on proteolysis and in quality of cheddar cheese. International Dairy Journal, 14, 591−97.

Valeur, N., Engel, P., Carbajal, N., Connolly, E., and Ladefoged, K. (2004). Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. Applied and Environmental Microbiology, 70, 1176-1181.

van Baarlen, P., Wells, J. M., and Kleerebezem, M. (2013). Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. Trends in Immunology, 34, 208-215.

van de Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S. D., and Maguin, E. (2002). Stress responses in lactic acid bacteria. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 82, 187-216.

van Der Heide, T., and Poolman, B. (2000). Osmoregulated ABC-transport system of *Lactococcus lactis* senses water stress via changes in the physical state of the membrane. Proceedings of the National Academy of Sciences of the United States of America, 97, 7102-7106.

van Pijkeren, J. P., Canchaya, C., Ryan, K. A., Li, Y., Claesson, M. J., Sheil, B., Steidler, L., O'mahony, L., Fitzgerald, G. F., Van Sinderen, D. and O'toole, P. W. (2006). Comparative and functional analysis of sortase-dependent proteins in the predicted secretome of *Lactobacillus salivarius* UCC118. Applied and Environmental Microbiology, 72, 4143-4153.

Vaughan, E. E., Heilig, H., Ben-Amor, K., and de Vos, W. M. (2005). Diversity, vitality and activities of intestinal lactic acid bacteria and bifidobacteria assessed by molecular approaches. FEMS Microbiology Reviews, 29, 477-490.

Velez, M. P., De Keersmaecker, S. C. J., and Vanderleyden, J. (2007). Adherence factors of *Lactobacillus* in the human gastrointestinal tract. FEMS Microbiology Letters, 276, 140-148.

Verges, M. C. C., Zuniga, M., Morel-Deville, F., Perez-Martinez, G., Zagorec, M., and Ehrlich, S. D. (1999). Relationships between arginine degradation, pH and survival in *Lactobacillus sakei*. FEMS Microbiology Letters, 180, 297-304.

Vesterlund, S., Paltta, J., Karp, M., and Ouwehand, A. C. (2005). Adhesion of bacteria to resected human colonic tissue: Quantitative analysis of bacterial adhesion and viability. Research in Microbiology, 156, 238-244.

Vintiñi, E. O., and Medina, M. (2014). Immune response in nasopharynx, lung, and blood elicited by experimental nasal pneumococcal vaccines containing live or heat-killed lactobacilli as mucosal adjuvants. Canadian Journal of Physiology and Pharmacology, 92, 1-8.

Volkov, A., Khoshnevis, S., Neumann, P., Herrfurth, C., Wohlwend, D., Ficner, R., and Feussner, I. (2013). Crystal structure analysis of a fatty acid double-bond hydratase from *Lactobacillus acidophilus*. Acta Crystallographica Section D-Biological Crystallography, 69, 648-657.

Volkov, A., Liavonchanka, A., Kamneva, O., Fiedler, T., Goebel, C., Kreikemeyer, B., Feussner, I. (2010). Myosin cross-reactive antigen of *Streptococcus pyogenes* M49 encodes a fatty acid double bond hydratase that plays a role in oleic acid detoxification and bacterial virulence. The Journal of Biological Chemistry. 285: 10353-10361.

Wall, T., Båth, K., Britton, R. A., Jonsson, H., Versalovic, J., and Roos, S. (2007). The early response to acid shock in *Lactobacillus reuteri* involves the ClpL chaperone and a putative cell wall-altering esterase. Applied and Environmental Microbiology, 73, 3924-3935.

Walter, J., Loach, D. M., Alqumber, M., Rockel, C., Hermann, C., Pfitzenmaier, M., and Tannock, G. W. (2007). D-alanyl ester depletion of teichoic acids in *Lactobacillus reuteri* 100-23 results in impaired colonization of the mouse gastrointestinal tract. Environmental Microbiology, 9, 1750-1760.

Wang, B., Wei, H., Yuan, J., Li, Q. R., Li, Y. S., Li, N., and Li, J. S. (2008). Identification of a surface protein from *Lactobacillus reuteri* JCM1081 that adheres to porcine gastric mucin and human enterocyte-like HT-29 cells. Current Microbiology, 57, 33-38.

Wang, J. C., Zhang, W. Y., Zhong, Z., Wei, A. B., Bao, Q. H., Zhang, Y., Sun, T. S., Postnikoff, A., Meng, H. and Zhang, H. P. (2012). Transcriptome analysis of probiotic *Lactobacillus casei* Zhang during fermentation in soymilk. Journal of Industrial Microbiology and Biotechnology, 39, 191-206.

Wang, S.-M., Zhang, L.-W., Fan, R.-B., Han, X., Yi, H.-X., Zhang, L.-L., Xue, C.-H., Li, H.-B., Zhang, Y.-H. and Shigwedha, N. (2014). Induction of HT-29 cells apoptosis by lactobacilli isolated from fermented products. Research in Microbiology, 165, 202-214.

Wang, Y., Corrieu, G., and Beal, C. (2005). Fermentation pH and temperature influence the cryotolerance of *Lactobacillus acidophilus* RD758. Journal of Dairy Science, 88, 21-29.

Weinrichter, B., Sollberger, H., Ginzinger, W., Jaros, D., and Rohm, H. (2004). Adjunct starter properties affect characteristic features of Swiss-type cheeses. Nahrung-Food, 48, 73-79.

Wickner, S., Maurizi, M. R., and Gottesman, S. (1999). Posttranslational quality control: folding, refolding, and degrading proteins. Science, 286, 1888-1893.

Williams, A. G., Withers, S. E., and Banks, J. M. (2000). Energy sources of non-starter lactic acid bacteria isolated from Cheddar cheese. International Dairy Journal, 10, 17-23.

Williams, W. L., Broquist, H. P. and Snell, E. E. (1947). Oleic acid and related compounds as growth factors for lactic acid bacteria. Journal of Biological Chemistry, 170, 619-630.

Wilson, R., Norris, E. L., Brachvogel, B., Angelucci, C., Zivkovic, S., Gordon, L., Bernardo, B. C., Stermann, J., Sekiguchi, K., Gorman, J. J. and Bateman, J. F. (2012). Changes in the chondrocyte and extracellular matrix proteome during post-natal mouse cartilage development. Molecular and Cellular Proteomics, 11, 1-18.

Wolvers, D., Antoine, J. M., Myllyluoma, E., Schrezenmeir, J., Szajewska, H., and Rijkers, G. T. (2010). Guidance for gubstantiating the evidence for beneficial effects of probiotics: prevention and anagement of infections by probiotics. Journal of Nutrition, 140, 698S-712S.

Woo, J., and Ahn, J. (2013). Probiotic-mediated competition, exclusion and displacement in biofilm formation by food-borne pathogens. Letters in Applied Microbiology, 56, 307-313.

Wouters, J. A., Kamphuis, H. H., Hugenholtz, J., Kuipers, O. P., De Vos, W. M., and Abee, T. (2000). Changes in glycolytic activity of *Lactococcus lactis* induced by low temperature. Applied and Environmental Microbiology, 66, 3686-3691.

Wu, C., Zhang, J., Du, G., and Chen, J. (2013). Aspartate protects *Lactobacillus casei* against acid stress. Applied Microbiology and Biotechnology, 97, 4083-4093.

Wu, C., Zhang, J., Wang, M., Du, G. and Chen, J. (2012). *Lactobacillus casei* combats acid stress by maintaining cell membrane functionality. Journal of Industrial Microbiology and Biotechnology, 39, 1031-1039.

Xi, X., Fan, J., Hou, Y., Gu, J., Shen, W., Li, Z., and Cui, Z. (2013). Characterization of three cryptic plasmids from *Lactobacillus plantarum* G63 that was isolated from Chinese pickle. Plasmid, 70, 321-328.

Xu, Y., and Kong, J. (2013). Construction and Potential Application of Controlled Autolytic Systems for *Lactobacillus casei* in Cheese Manufacture. Journal of Food Protection, 76, 1187-1193.

Yadav, A. K., Tyagi, A., Kaushik, J. K., Saklani, A. C., Grover, S., and Batish, V. K. (2013). Role of surface layer collagen binding protein from indigenous *Lactobacillus plantarum* 91 in adhesion and its anti-adhesion potential against gut pathogen. Microbiological Research, 168, 639-645.

Younes, H., Coudray, C., Bellanger, J., Demigne, C., Rayssiguier, Y., and Remesy, C. (2001). Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. British Journal of Nutrition, 86, 479-485.

Zagato, E., Mileti, E., Massimiliano, L., Fasano, F., Budelli, A., Penna, G., and Rescigno, M. (2014). *Lactobacillus paracasei* CBA L74 metabolic products and fermented milk for infant formula have anti-inflammatory activity on dendritic cells in vitro and protective effects against colitis and an enteric pathogen in vivo. PLoS ONE, 9, e87615.

Zhai, Z., Douillard, F. P., An, H., Wang, G., Guo, X., Luo, Y., and Hao, Y. (2013). Proteomic characterization of the acid tolerance response in *Lactobacillus delbrueckii* subsp. *bulgaricus* CAUH1 and functional identification of a novel acid stress-related transcriptional regulator Ldb0677. Environmental Microbiology, 16, 1524–1537.

Zhang, W., Wang, H., Liu, J., Zhao, Y., Gao, K., and Zhang, J. (2013). Adhesive ability means inhibition activities for *Lactobacillus* against pathogens and S-layer protein plays an important role in adhesion. Anaerobe, 22, 97-103.

Zhang, W., Yu, D., Sun, Z., Wu, R., Chen, X., Chen, W., Meng, H., Hu, S., Zhang, H. (2010). Complete genome sequence of *Lactobacillus casei* Zhang a new probiotic strain isolated from traditional homemade koumiss in Inner Mongolia, China. Journal of Bacteriology, 192, 5268-5269.

Zhu, Y., Zhang, Y. P. and Li, Y. (2009). Understanding the industrial application potential of lactic acid bacteria through genomics. Applied Microbiology and Biotechnology, 83, 597-610.

## Appendix A

## Impact of lactose starvation on the physiology of *Lactobacillus casei* GCRL163 in the presence or absence of Tween 80

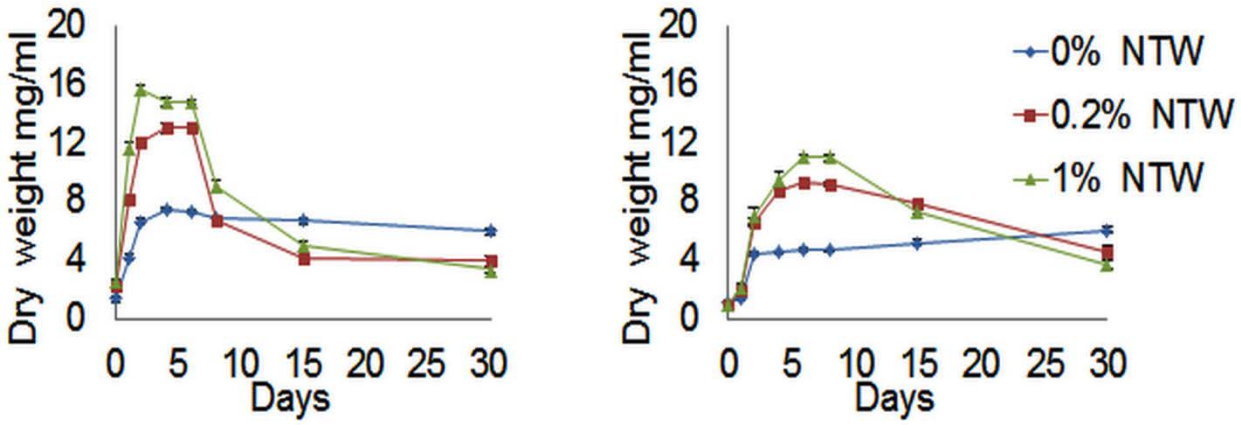


**Figure A1:** Dry weight of *Lactobacillus casei* GCRL163 in modified buffered MRS broth. Containing 0%, 0.2% or 1% lactose and either supplemented with Tween 80 or lacking Tween 80.

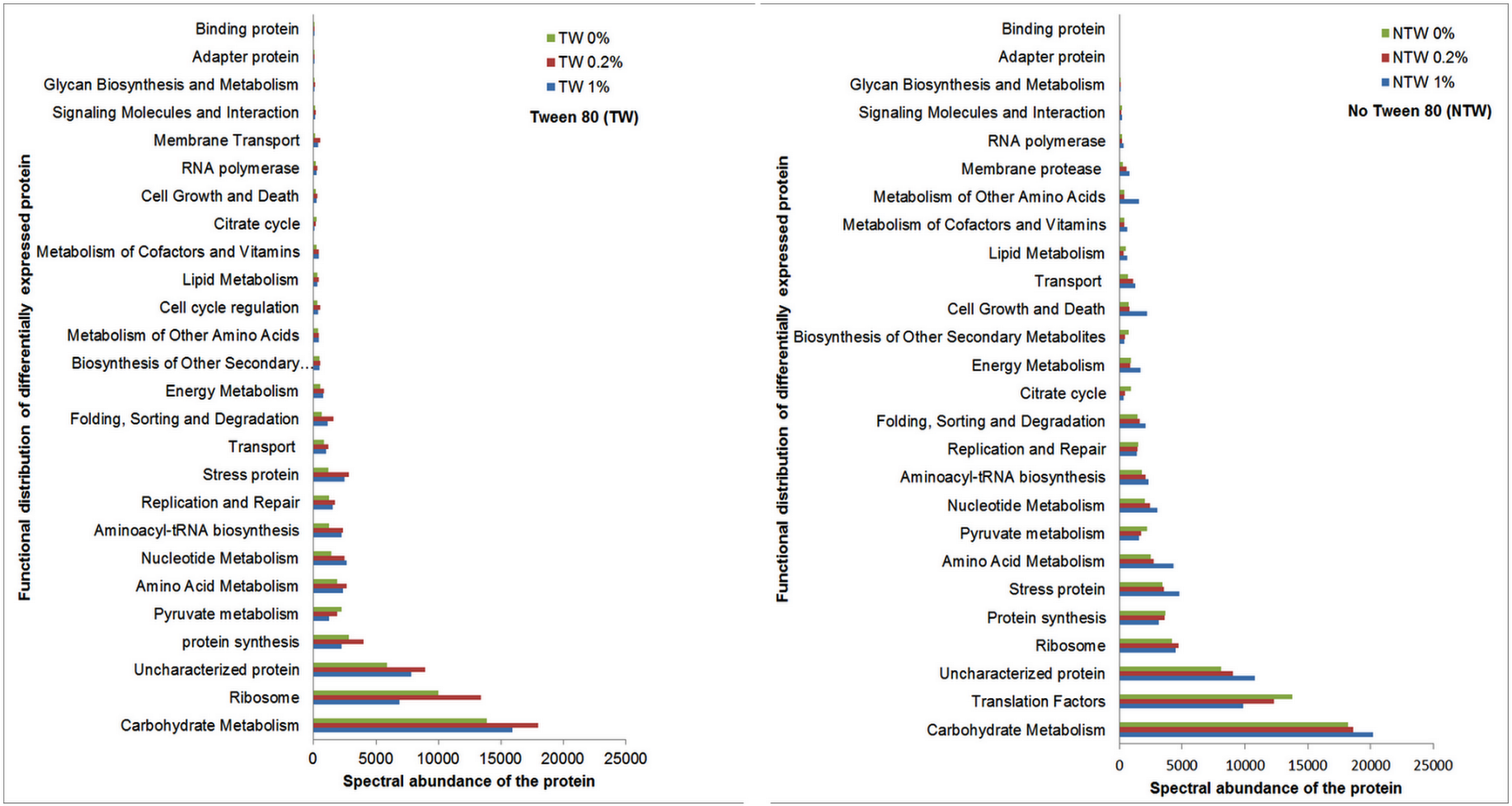


**Figure A2:** Distribution of identified protein groups associated with their functional groups of *Lactobacillus casei* GCRL163 identified in the study.

Table A1: Proteomics data of *Lactobacillus casei* GCRL163with in mMRS with present of Tween 80 with three levels of lactose (0%, 0.2% or 1%).

**TW = Tween 80**
**1% = 1% Lactose**
**0.2% = 0.2% Lactose**
**0% = 0% Lactose**

| Pathway | Protein Name | *P*-Value TW 0.2 vs 1 | Fold Change | Log 2 Fold change | *P*-Value TW 0 vs 1 | Fold Change | Log 2 Fold change |
|---|---|---|---|---|---|---|---|
| ABC transporters | Extracellular solute-binding | 0.16 | 1.02 | 0.02 | 0.00 | 0.06 | -4.04 |
| ABC transporters | Cell division ATP-binding protein FtsE | 0.00 | 3.93 | 1.98 | 0.03 | 2.60 | 1.38 |
| ABC transporters | MalK1 | 0.21 | 2.33 | 1.22 | 0.00 | 5.27 | 2.40 |
| ABC transporters | MalK2 | 0.04 | 3.09 | 1.63 | 0.20 | 0.65 | -0.62 |
| ABC transporters | Glutamine-binding protein | 0.04 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Alanine, aspartate and glutamate metabolism | Glutamine synthetase (EC 6.3.1.2) | 0.03 | 0.95 | -0.07 | 0.02 | 0.63 | -0.67 |
| Alanine, aspartate and glutamate metabolism | Glutamine synthetase (EC 6.3.1.2) | 0.03 | 0.95 | -0.07 | 0.02 | 0.63 | -0.67 |
| Alanine, aspartate and glutamate metabolism | Asparagine synthetase (EC 6.3.5.4) | 0.20 | 0.81 | -0.31 | 0.01 | 0.30 | -1.73 |
| Alanine, aspartate and glutamate metabolism | Glutamine synthetase (EC 6.3.1.2) | 0.02 | 0.93 | -0.11 | 0.02 | 0.62 | -0.68 |
| Alanine, aspartate and glutamate metabolism | Alanine dehydrogenase (EC 1.4.1.1) | 0.82 | 1.52 | 0.60 | 0.00 | 2.68 | 1.42 |
| Alanine, aspartate and glutamate metabolism | Alanine dehydrogenase (EC 1.4.1.1) | 0.31 | 2.22 | 1.15 | 0.01 | 3.09 | 1.63 |
| Alanine, aspartate and glutamate metabolism | Alanine dehydrogenase (EC 1.4.1.1) | 0.31 | 2.22 | 1.15 | 0.01 | 3.09 | 1.63 |
| Amino sugar and nucleotide sugar metabolism | 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase | 0.03 | 0.96 | -0.06 | 0.87 | 0.91 | -0.14 |
| Amino sugar and nucleotide sugar metabolism | Glucosamine-6-phosphate deaminase (EC 3.5.99.6) | 0.04 | 0.66 | -0.59 | 0.01 | 0.28 | -1.82 |
| Amino sugar and nucleotide sugar metabolism | YbfG | 0.00 | 3.67 | 1.87 | 0.02 | 2.33 | 1.22 |
| Amino sugar and nucleotide sugar metabolism | YbfG | 0.00 | 3.67 | 1.87 | 0.02 | 2.33 | 1.22 |
| Amino sugar and nucleotide sugar metabolism | L-glutamine--D-fructose-6-phosphate amidotransferase | 0.19 | 1.07 | 0.10 | 0.00 | 2.04 | 1.03 |
| Amino sugar and nucleotide sugar metabolism | Glucosamine-6-phosphate deaminase (EC 3.5.99.6) | 0.07 | 0.76 | -0.40 | 0.01 | 0.27 | -1.87 |
| Amino sugar and nucleotide sugar metabolism | Glucosamine-6-phosphate deaminase (EC 3.5.99.6) | 0.07 | 0.76 | -0.40 | 0.01 | 0.27 | -1.87 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Amino sugar and nucleotide sugar metabolism | NAD-dependent epimerase/dehydratase | 0.10 | 0.66 | -0.60 | 0.00 | 0.32 | -1.65 |
| Amino sugar and nucleotide sugar metabolism | NAD-dependent epimerase/dehydratase | 0.03 | 0.54 | -0.88 | 0.51 | 0.75 | -0.42 |
| Aminoacyl-tRNA biosynthesis | Tyrosyl-tRNA synthetase (EC 6.1.1.1) | 0.01 | 3.44 | 1.78 | 0.20 | 0.65 | -0.62 |
| Aminoacyl-tRNA biosynthesis | Aspartyl-tRNA synthetase (EC 6.1.1.12) | 0.02 | 0.71 | -0.49 | 0.00 | 0.22 | -2.17 |
| Aminoacyl-tRNA biosynthesis | Phenylalanyl-tRNA synthetase beta chain (EC 6.1.1.20) | 0.03 | 0.46 | -1.12 | 0.02 | 0.42 | -1.24 |
| Aminoacyl-tRNA biosynthesis | Phenylalanyl-tRNA synthetase beta chain (EC 6.1.1.20) | 0.03 | 0.45 | -1.14 | 0.02 | 0.41 | -1.28 |
| Aminoacyl-tRNA biosynthesis | Phenylalanyl-tRNA synthetase alpha chain (EC 6.1.1.20) | 0.02 | 0.45 | -1.15 | 0.00 | 0.29 | -1.77 |
| Aminoacyl-tRNA biosynthesis | Alanyl-tRNA synthetase (EC 6.1.1.7) | 0.15 | 0.95 | -0.07 | 0.00 | 0.32 | -1.64 |
| Aminoacyl-tRNA biosynthesis | Leucyl-tRNA synthetase (EC 6.1.1.4) (Leucine--tRNA ligase) (LeuRS) | 0.16 | 0.91 | -0.14 | 0.04 | 0.54 | -0.88 |
| Aminoacyl-tRNA biosynthesis | Asparaginyl-tRNA synthetase (EC 6.1.1.22) (Asparagine--tRNA ligase) | 0.75 | 1.21 | 0.28 | 0.03 | 0.53 | -0.91 |
| Aminoacyl-tRNA biosynthesis | Threonyl-tRNA synthetase (EC 6.1.1.3) (Threonine--tRNA ligase) (ThrRS) | 0.14 | 1.04 | 0.05 | 0.00 | 0.51 | -0.97 |
| Aminoacyl-tRNA biosynthesis | Seryl-tRNA synthetase (EC 6.1.1.11) (Serine--tRNA ligase) | 0.66 | 1.16 | 0.21 | 0.00 | 0.37 | -1.43 |
| Aminoacyl-tRNA biosynthesis | Leucyl-tRNA synthetase (EC 6.1.1.4) (Leucine--tRNA ligase) | 0.16 | 0.91 | -0.14 | 0.04 | 0.54 | -0.88 |
| Aminoacyl-tRNA biosynthesis | Threonyl-tRNA synthetase | 0.14 | 1.04 | 0.05 | 0.00 | 0.51 | -0.97 |
| Aminoacyl-tRNA biosynthesis | Leucyl-tRNA synthetase (EC 6.1.1.4) | 0.16 | 0.91 | -0.14 | 0.04 | 0.54 | -0.88 |
| Aminoacyl-tRNA biosynthesis | Asparaginyl-tRNA synthetase (EC 6.1.1.22) | 0.75 | 1.21 | 0.28 | 0.03 | 0.53 | -0.91 |
| Aminoacyl-tRNA biosynthesis | Seryl-tRNA synthetase (EC 6.1.1.11) | 0.44 | 1.06 | 0.09 | 0.00 | 0.35 | -1.52 |
| Aminoacyl-tRNA biosynthesis | Threonyl-tRNA synthetase (EC 6.1.1.3) | 0.14 | 1.04 | 0.05 | 0.00 | 0.51 | -0.97 |
| Aminoacyl-tRNA biosynthesis | Glycyl-tRNA synthetase beta subunit (EC 6.1.1.14) | 0.08 | 0.88 | -0.19 | 0.00 | 0.41 | -1.28 |
| Aminoacyl-tRNA biosynthesis | Glycyl-tRNA synthetase beta subunit (EC 6.1.1.14) | 0.08 | 0.88 | -0.19 | 0.00 | 0.41 | -1.28 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aminoacyl-tRNA biosynthesis | Glycyl-tRNA synthetase beta subunit (EC 6.1.1.14) | 0.08 | 0.88 | -0.19 | 0.00 | 0.41 | -1.28 |
| Aminoacyl-tRNA biosynthesis | Seryl-tRNA synthetase (EC 6.1.1.11) | 0.49 | 1.07 | 0.10 | 0.00 | 0.24 | -2.04 |
| Aminoacyl-tRNA biosynthesis | Histidyl-tRNA synthetase (EC 6.1.1.21) | 0.54 | 1.05 | 0.07 | 0.00 | 0.29 | -1.78 |
| Aminoacyl-tRNA biosynthesis | Histidyl-tRNA synthetase (EC 6.1.1.21) | 0.54 | 1.05 | 0.07 | 0.00 | 0.29 | -1.78 |
| Aminoacyl-tRNA biosynthesis | Histidyl-tRNA synthetase (EC 6.1.1.21) (Histidine--tRNA ligase) (HisRS) | 0.54 | 1.05 | 0.07 | 0.00 | 0.29 | -1.78 |
| Aminoacyl-tRNA biosynthesis | Phenylalanyl-tRNA synthetase (Beta subunit) (EC 6.1.1.20) | 0.91 | 1.17 | 0.23 | 0.03 | 0.40 | -1.31 |
| Aminoacyl-tRNA biosynthesis | Leucyl-tRNA synthetase (Fragment) | 0.29 | 0.81 | -0.30 | 0.00 | 0.35 | -1.52 |
| Aminoacyl-tRNA biosynthesis | S-adenosylmethionine:tRNA ribosyltransferase-isomerase | 0.19 | 0.78 | -0.36 | 0.00 | 0.27 | -1.87 |
| Aminoacyl-tRNA biosynthesis | S-adenosylmethionine:tRNA ribosyltransferase-isomerase | 0.19 | 0.78 | -0.36 | 0.00 | 0.27 | -1.87 |
| Apoptosis | Superfamily II DNA and RNA helicase | 0.83 | 1.10 | 0.14 | 0.03 | 0.38 | -1.38 |
| Arginine and proline metabolism | Oxaloacetate decarboxylase | 0.03 | 2.07 | 1.05 | 0.01 | 2.33 | 1.22 |
| Arginine and proline metabolism | Oxaloacetate decarboxylase | 0.00 | 23.43 | 4.55 | 0.00 | 37.92 | 5.24 |
| Arginine and proline metabolism | Dipeptidase A, Cysteine peptidase | 0.47 | 1.54 | 0.63 | 0.01 | 2.22 | 1.15 |
| Arginine and proline metabolism | Dipeptidase D-type | 0.41 | 1.61 | 0.69 | 0.00 | 2.70 | 1.43 |
| Arginine and proline metabolism | Dipeptidase (EC 3.4.-.-) | 0.06 | 0.70 | -0.52 | 0.00 | 0.29 | -1.78 |
| Arginine and proline metabolism | Dipeptidase | 0.13 | 0.76 | -0.39 | 0.01 | 0.34 | -1.54 |
| Arginine and proline metabolism | Dipeptidase, peptidase U 34 family | 0.06 | 0.70 | -0.52 | 0.00 | 0.29 | -1.78 |
| Arginine and proline metabolism | Dipeptidase A | 0.19 | 0.78 | -0.36 | 0.03 | 0.42 | -1.26 |
| Arginine and proline metabolism | N utilization substance protein B | 0.26 | 0.74 | -0.43 | 0.03 | 0.48 | -1.05 |
| Arginine and proline metabolism | N utilization substance | 0.26 | 0.74 | -0.43 | 0.03 | 0.48 | -1.05 |
| Arginine and proline metabolism | PepS aminopeptidase, Metallo peptidase, MEROPS family M29 | 0.04 | 2.42 | 1.27 | 0.87 | 1.00 | 0.00 |
| Ascorbate and aldarate metabolism | NADH peroxidase (EC 1.11.1.1) | 0.00 | 0.42 | -1.26 | 0.00 | 0.30 | -1.74 |
| binding protein | Adenine/guanine phosphoribosyltransferase related PRPP | 0.02 | 0.54 | -0.89 | 0.03 | 0.45 | -1.16 |
| binding protein | Pur operon repressor | 0.02 | 0.52 | -0.96 | 0.02 | 0.43 | -1.20 |
| Butanoate metabolism | Butanol dehydrogenase | 0.20 | 1.53 | 0.62 | 0.02 | 2.07 | 1.05 |

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|---|---|---|---|---|---|---|---|
| Cell cycle | Cell-division initiation protein (Septum placement) | 0.27 | 1.69 | 0.76 | 0.03 | 1.59 | 0.67 |
| Cell division protein | Cell division initiation protein | 0.27 | 1.69 | 0.76 | 0.03 | 1.59 | 0.67 |
| Cell division protein | Cell division protein ftsA | 0.04 | 0.62 | -0.69 | 0.07 | 0.49 | -1.02 |
| Cell division protein | Cell division protein SufI | 0.02 | 0.46 | -1.13 | 0.33 | 0.66 | -0.60 |
| Chaperones and folding catalysts | Aluminum resistance protein | 0.19 | 0.85 | -0.24 | 0.04 | 0.44 | -1.17 |
| Citrate cycle | Putative citrate lyase alpha subunit (EC 4.1.3.6) (EC 2.8.3.10) | 0.02 | 9.51 | 3.25 | 0.00 | 12.39 | 3.63 |
| Citrate cycle | Citrate lyase, beta subunit (EC 4.1.3.6) | 0.02 | 12.93 | 3.69 | 0.00 | 22.79 | 4.51 |
| Citrate cycle | Citrate lyase, alpha subunit | 0.02 | 9.51 | 3.25 | 0.00 | 12.34 | 3.63 |
| Cold shock protein | Cold shock protein A (Cold-shock protein) | 0.01 | 0.62 | -0.68 | 0.01 | 1.51 | 0.60 |
| Cyanoamino acid metabolism | Beta-glucoside operon antiterminator | 0.44 | 1.50 | 0.59 | 0.00 | 0.10 | -3.33 |
| Cysteine and methionine metabolism | S-adenosylmethionine synthase (AdoMet synthase) (EC 2.5.1.6) | 0.01 | 0.60 | -0.73 | 0.01 | 0.39 | -1.37 |
| Cysteine and methionine metabolism | S-adenosylmethionine synthase (AdoMet synthase) (EC 2.5.1.6) | 0.01 | 0.60 | -0.73 | 0.01 | 0.39 | -1.37 |
| Cysteine and methionine metabolism | Cystathionine beta-lyase family protein | 0.19 | 0.85 | -0.24 | 0.04 | 0.44 | -1.17 |
| Cysteine and methionine metabolism | Cystathionine beta-lyase family protein | 0.20 | 0.82 | -0.28 | 0.02 | 0.34 | -1.54 |
| Cysteine and methionine metabolism | Cysteine synthase (EC 2.5.1.47) | 0.15 | 0.96 | -0.06 | 0.00 | 0.03 | -5.00 |
| Cysteine and methionine metabolism | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14) | 0.07 | 0.59 | -0.76 | 0.01 | 0.38 | -1.38 |
| Cysteine and methionine metabolism | Pyrrolidone-carboxylate peptidase (EC 3.4.19.3) | 0.04 | 2.81 | 1.49 | 0.32 | 1.39 | 0.47 |
| D-Alanine metabolism | D-alanine--D-alanine ligase (EC 6.3.2.4) (D-Ala-D-Ala ligase) (D-alanylalanine synthetase) | 0.22 | 1.72 | 0.78 | 0.01 | 1.84 | 0.88 |
| D-Glutamine and D-glutamate metabolism | Uncharacterized protein | 0.76 | 1.15 | 0.20 | 0.04 | 0.39 | -1.35 |
| Dioxin degradation | 4-oxalocrotonate tautomerase | 0.02 | 2.60 | 1.38 | 0.00 | 2.60 | 1.38 |
| Dioxin degradation | Putative 4-oxalocrotonate tautomerase 4-OT (EC 5.3.2.-) | 0.02 | 2.60 | 1.38 | 0.00 | 2.60 | 1.38 |
| DNA replication | DNA gyrase, A subunit (EC 5.99.1.3) | 0.11 | 0.89 | -0.17 | 0.04 | 0.52 | -0.93 |
| DNA replication | DNA gyrase, A subunit | 0.11 | 0.89 | -0.17 | 0.04 | 0.52 | -0.93 |

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|---|---|---|---|---|---|---|---|
| DNA replication proteins | DNA gyrase subunit B (EC 5.99.1.3) | 0.26 | 0.90 | -0.15 | 0.00 | 0.28 | -1.85 |
| Energy Metabolism | Predicted hydrolase of HD superfamily | 0.13 | 0.84 | -0.25 | 0.01 | 0.35 | -1.50 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100) | 0.78 | 1.09 | 0.13 | 0.00 | 0.35 | -1.52 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100) | 0.78 | 1.09 | 0.13 | 0.00 | 0.35 | -1.52 |
| Fatty acid biosynthesis | Acyl carrier protein 2 (ACP 2) | 0.62 | 1.32 | 0.40 | 0.00 | 0.17 | -2.54 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier-protein] synthase II (EC 2.3.1.41) | 0.88 | 3.56 | 1.83 | 0.00 | 0.20 | -2.32 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier-protein] synthase 2 (EC 2.3.1.179) | 1.00 | 3.29 | 1.72 | 0.00 | 0.16 | -2.60 |
| Fatty acid biosynthesis | Fatty acid/phospholipid biosynthesis enzyme | 0.49 | 1.52 | 0.60 | 0.03 | 0.48 | -1.05 |
| Fatty acid biosynthesis | [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39) | 0.03 | 3.67 | 1.87 | 1.00 | 1.00 | 0.00 |
| Fatty acid biosynthesis | Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39) | 0.03 | 3.40 | 1.77 | 1.00 | 1.00 | 0.00 |
| Fatty acid metabolism | Acetyl-CoA carboxylase, biotin carboxyl carrier protein | 0.03 | 5.00 | 2.32 | 1.00 | 1.00 | 0.00 |
| Fatty acid metabolism | Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta | 0.03 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Fatty acid metabolism | Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta | 0.03 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Fructose and mannose metabolism | PTS system D-fructose-specific IIC component | 0.75 | 1.36 | 0.45 | 0.00 | 0.27 | -1.87 |
| Fructose and mannose metabolism | 1-phosphofructokinase (Fructose-1-phosphate kinase) (EC 2.7.1.56) | 0.69 | 1.41 | 0.49 | 0.00 | 0.19 | -2.40 |
| Galactose metabolism | Tagatose-6-phosphate kinase | 0.56 | 1.48 | 0.56 | 0.00 | 0.22 | -2.16 |
| Galactose metabolism | LacE | 0.58 | 1.47 | 0.55 | 0.00 | 0.35 | -1.52 |
| Galactose metabolism | LacE | 0.58 | 1.47 | 0.55 | 0.00 | 0.35 | -1.52 |
| Galactose metabolism | Alpha-glucosidase (EC 3.2.1.20) | 0.03 | 0.75 | -0.42 | 0.12 | 1.25 | 0.32 |
| Galactose metabolism | Nucleoside-diphosphate-sugar epimerase | 0.03 | 0.54 | -0.88 | 0.38 | 0.70 | -0.52 |
| Galactose metabolism | Galactokinase (EC 2.7.1.6) (Galactose kinase) | 0.02 | 1.72 | 0.78 | 0.00 | 2.91 | 1.54 |
| Galactose metabolism | Galactose-6-phosphate isomerase lacB subunit (EC 5.3.1.26) | 0.01 | 0.81 | -0.31 | 0.00 | 0.06 | -3.96 |

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|---|---|---|---|---|---|---|---|
| Galactose metabolism | Galactose-6-phosphate isomerase lacA subunit (EC 5.3.1.26) | 0.00 | 0.68 | -0.56 | 0.00 | 0.05 | -4.35 |
| Galactose metabolism | Aldo/keto reductase related enzyme | 0.01 | 0.71 | -0.50 | 0.01 | 0.48 | -1.07 |
| Galactose metabolism | 6-phospho-beta-galactosidase (EC 3.2.1.85) | 0.05 | 0.95 | -0.08 | 0.00 | 0.19 | -2.36 |
| Galactose metabolism | Galactose-6-phosphate isomerase subunit lacB (EC 5.3.1.26) | 0.01 | 0.80 | -0.32 | 0.00 | 0.06 | -4.00 |
| Galactose metabolism | Galactose-6-phosphate isomerase subunit lacA (EC 5.3.1.26) | 0.00 | 0.68 | -0.56 | 0.00 | 0.05 | -4.35 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 2 (EC 4.1.2.40) | 0.00 | 0.72 | -0.47 | 0.00 | 0.56 | -0.83 |
| Galactose metabolism | Galactokinase 1 (EC 2.7.1.6) (Galactose kinase 1) | 0.04 | 1.74 | 0.80 | 0.00 | 2.85 | 1.51 |
| Galactose metabolism | Galactokinase (EC 2.7.1.6) (Galactose kinase) | 0.04 | 1.74 | 0.80 | 0.00 | 2.85 | 1.51 |
| Galactose metabolism | Galactose 6-phosphate isomerase | 0.00 | 0.66 | -0.59 | 0.00 | 0.05 | -4.28 |
| Galactose metabolism | Sugar-phosphate isomerase, RpiB/LacA/LacB family | 0.00 | 0.66 | -0.59 | 0.00 | 0.05 | -4.28 |
| Galactose metabolism | Lactose phosphotransferase system repressor | 0.02 | 0.81 | -0.31 | 0.00 | 0.07 | -3.79 |
| Galactose metabolism | Galactokinase (EC 2.7.1.6) (Galactose kinase) | 0.05 | 1.75 | 0.81 | 0.00 | 2.88 | 1.53 |
| Galactose metabolism | UDP-glucose 4-epimerase (EC 5.1.3.2) (Galactowaldenase) (UDP-galactose 4-epimerase) | 0.23 | 1.46 | 0.55 | 0.00 | 2.09 | 1.06 |
| Galactose metabolism | Galactose-1-phosphate uridylyltransferase (Gal-1-P uridylyltransferase) (EC 2.7.7.12) | 0.67 | 1.40 | 0.48 | 0.03 | 1.34 | 0.42 |
| Galactose metabolism | UDP-galactose 4-epimerase (EC 5.1.3.2) | 0.05 | 1.60 | 0.67 | 0.00 | 2.46 | 1.30 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) | 0.35 | 1.15 | 0.21 | 0.00 | 0.18 | -2.45 |
| Galactose metabolism | Lactose-specific phosphotransferase enzyme IIA component | 0.50 | 1.20 | 0.26 | 0.00 | 0.11 | -3.12 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) | 0.25 | 1.12 | 0.16 | 0.00 | 0.19 | -2.37 |

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|---|---|---|---|---|---|---|---|
| Galactose metabolism | Aldo/keto reductase | 0.86 | 1.34 | 0.42 | 0.04 | 0.52 | -0.94 |
| Galactose metabolism | LacG | 0.14 | 1.11 | 0.15 | 0.00 | 0.11 | -3.17 |
| Galactose metabolism | Galactose-1-phosphate uridylyltransferase 1 (Gal-1-P uridylyltransferase 1) | 0.67 | 1.40 | 0.48 | 0.03 | 1.34 | 0.42 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) | 0.34 | 1.15 | 0.21 | 0.00 | 0.19 | -2.41 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) | 0.34 | 1.13 | 0.18 | 0.00 | 0.20 | -2.35 |
| Galactose metabolism | Uncharacterized protein | 0.16 | 1.11 | 0.15 | 0.00 | 0.12 | -3.09 |
| Galactose metabolism | Lactose-specific phosphotransferase enzyme IIA component | 0.50 | 1.20 | 0.26 | 0.00 | 0.11 | -3.12 |
| Galactose metabolism | Tagatose-6-phosphate kinase (Phosphotagatokinase) (EC 2.7.1.144) | 0.82 | 1.35 | 0.43 | 0.00 | 0.10 | -3.33 |
| Galactose metabolism | Tagatose-6-phosphate kinase (EC 2.7.1.144) | 0.95 | 1.31 | 0.39 | 0.00 | 0.10 | -3.25 |
| Galactose metabolism | Aldo/keto reductase of diketogulonate reductase family | 0.05 | 0.80 | -0.32 | 0.00 | 0.14 | -2.87 |
| Galactose metabolism | Tagatose-6-phosphate kinase | 0.97 | 1.31 | 0.39 | 0.00 | 0.07 | -3.84 |
| Galactose metabolism | Aldo/keto reductase of diketogulonate reductase family | 0.12 | 0.87 | -0.19 | 0.00 | 0.12 | -3.08 |
| Galactose metabolism | Aldo/keto reductase (Fragment) | 0.19 | 0.87 | -0.20 | 0.00 | 0.16 | -2.60 |
| Galactose metabolism | Aldo/keto reductase (Fragment) | 0.19 | 0.87 | -0.20 | 0.00 | 0.16 | -2.60 |
| Galactose metabolism | Aldo/keto reductase (Fragment) | 0.07 | 0.77 | -0.38 | 0.00 | 0.15 | -2.78 |
| Galactose metabolism | Aldo/keto reductase (Fragment) | 0.07 | 0.77 | -0.38 | 0.00 | 0.15 | -2.78 |
| Galactose metabolism | Cellobiose PTS, EIIB (EC 2.7.1.69) | 0.36 | 1.89 | 0.92 | 0.04 | 2.04 | 1.03 |
| Galactose metabolism | Fructose-specific phosphotransferase system, enzyme IIABC (EC 2.7.1.69) | 0.89 | 1.30 | 0.38 | 0.00 | 0.22 | -2.16 |
| Galactose metabolism | Lactose-specific IIC component | 0.58 | 1.47 | 0.55 | 0.00 | 0.35 | -1.52 |
| Galactose metabolism | PTS system lactose-specific EIICB component (EIICB-Lac) | 0.58 | 1.47 | 0.55 | 0.00 | 0.35 | -1.52 |
| Galactose metabolism | UDP-glucose 4-epimerase (EC 5.1.3.2) | 0.03 | 0.54 | -0.88 | 0.51 | 0.75 | -0.42 |
| Galactose metabolism | Nucleoside-diphosphate-sugar epimerase | 0.03 | 0.54 | -0.88 | 0.51 | 0.75 | -0.42 |
| Glycerolipid metabolism | Phosphate acyltransferase (EC 2.3.1.n2) | 0.49 | 1.52 | 0.60 | 0.03 | 0.48 | -1.05 |

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| Glycerolipid metabolism | Phosphate acyltransferase (EC 2.3.1.n2) | 0.49 | 1.52 | 0.60 | 0.03 | 0.48 | -1.05 |
| Glycerolipid metabolism | Esterase/lipase | 0.00 | 0.21 | -2.24 | 0.00 | 0.21 | -2.24 |
| Glycine, serine and threonine metabolism | Phosphoglycerate dehydrogenase | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |
| Glycine, serine and threonine metabolism | Aspartate semialdehyde dehydrogenase (EC 1.2.1.11) | 0.04 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| Glycine, serine and threonine metabolism | Aspartate semialdehyde dehydrogenase | 0.04 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| Glycine, serine and threonine metabolism | Aspartate-semialdehyde dehydrogenase | 0.04 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| Glycolysis / Gluconeogenesis | NADPH:quinone reductase related Zn-dependent oxidoreductase | 1.00 | 1.00 | 0.00 | 0.04 | 1.80 | 0.85 |
| Glycolysis / Gluconeogenesis | Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) | 0.03 | 0.52 | -0.94 | 0.73 | 0.82 | -0.28 |
| Glycolysis / Gluconeogenesis | 2,5-diketo-D-gluconate reductase (EC 1.1.1.274) | 0.00 | 0.69 | -0.54 | 0.00 | 0.44 | -1.18 |
| Glycolysis / Gluconeogenesis | Phosphoglycerate kinase (EC 2.7.2.3) | 0.04 | 0.89 | -0.17 | 0.01 | 0.52 | -0.93 |
| Glycolysis / Gluconeogenesis | Pyruvate kinase (EC 2.7.1.40) | 0.00 | 0.84 | -0.25 | 0.01 | 0.69 | -0.54 |
| Glycolysis / Gluconeogenesis | GalM | 0.03 | 2.69 | 1.43 | 0.00 | 3.90 | 1.96 |
| Glycolysis / Gluconeogenesis | L-lactate dehydrogenase (L-LDH) (EC 1.1.1.27) | 0.00 | 0.81 | -0.31 | 0.02 | 0.68 | -0.56 |
| Glycolysis / Gluconeogenesis | Aldose 1-epimerase | 0.02 | 3.10 | 1.63 | 0.01 | 4.53 | 2.18 |
| Glycolysis / Gluconeogenesis | Mutarotase (Fragment) | 0.01 | 3.26 | 1.70 | 0.01 | 4.28 | 2.10 |
| Glycolysis / Gluconeogenesis | Uncharacterized protein | 0.00 | 0.81 | -0.31 | 0.02 | 0.68 | -0.56 |
| Glycolysis / Gluconeogenesis | Phosphoglycerate kinase (EC 2.7.2.3) | 0.04 | 0.89 | -0.17 | 0.01 | 0.52 | -0.93 |
| Glycolysis / Gluconeogenesis | 6-phospho-beta-glucosidase | 0.05 | 2.48 | 1.31 | 0.04 | 2.04 | 1.03 |
| Glycolysis / Gluconeogenesis | Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) | 0.40 | 1.14 | 0.19 | 0.00 | 0.41 | -1.28 |
| Glycolysis / Gluconeogenesis | Glyceraldehyde 3-phosphate dehydrogenase | 0.05 | 0.84 | -0.26 | 0.01 | 0.57 | -0.81 |
| Glycolysis / Gluconeogenesis | Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (2-phosphoglycerate dehydratase) | 0.37 | 1.13 | 0.18 | 0.00 | 0.38 | -1.41 |
| Glycolysis / Gluconeogenesis | Pyruvate dehydrogenase complex, E1 component, alpha subunit (EC 1.2.4.1) | 0.12 | 1.75 | 0.81 | 0.00 | 1.62 | 0.70 |

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|---|---|---|---|---|---|---|---|
| Glycolysis / Gluconeogenesis | Acetoin-pyruvate dihydrolipoamide acetyltransferase | 0.06 | 2.52 | 1.33 | 0.02 | 1.64 | 0.72 |
| Glycolysis / Gluconeogenesis | Oxidoreductase | 0.05 | 0.80 | -0.32 | 0.00 | 0.14 | -2.87 |
| Glycolysis / Gluconeogenesis | Putative phosphotransferase LCABL 17380 (EC 2.7.-.-) | 0.29 | 2.33 | 1.22 | 0.04 | 2.04 | 1.03 |
| Glycolysis / Gluconeogenesis | Putative phosphotransferase LSEI 1522 (EC 2.7.-.-) | 0.29 | 2.33 | 1.22 | 0.04 | 2.04 | 1.03 |
| Glycolysis / Gluconeogenesis | Phosphotransferase system | 0.07 | 0.80 | -0.33 | 0.00 | 0.13 | -2.99 |
| Glycolysis / Gluconeogenesis | Fusion of IIA, IIB and IIC component of mannitol | 0.89 | 1.30 | 0.38 | 0.00 | 0.22 | -2.16 |
| Glycolysis / Gluconeogenesis | PTS system, lactose-specific IIBC component | 0.58 | 1.47 | 0.55 | 0.00 | 0.35 | -1.52 |
| Glycolysis / Gluconeogenesis | PTS system, lactose-specific IIBC component | 0.47 | 1.56 | 0.64 | 0.00 | 0.35 | -1.52 |
| Glycolysis / Gluconeogenesis | Fructose-1,6-bisphosphatase class 3 | 0.20 | 2.33 | 1.22 | 0.00 | 5.00 | 2.32 |
| Glycolysis / Gluconeogenesis | Fructose-1,6-bisphosphatase class 3 | 0.20 | 2.33 | 1.22 | 0.00 | 5.00 | 2.32 |
| Glycolysis / Gluconeogenesis | Fructose-1,6-bisphosphatase class 3 | 0.20 | 2.07 | 1.05 | 0.00 | 4.20 | 2.07 |
| Glycolysis / Gluconeogenesis | L-lactate dehydrogenase 2 (L-LDH 2) (EC 1.1.1.27) | 1.00 | 1.00 | 0.00 | 0.00 | 4.20 | 2.07 |
| Glycolysis / Gluconeogenesis | L-lactate dehydrogenase 1 (L-LDH 1) (EC 1.1.1.27) | 1.00 | 1.00 | 0.00 | 0.00 | 3.40 | 1.77 |
| Glycolysis / Gluconeogenesis | Triosephosphate isomerase (TIM) (EC 5.3.1.1) (Triose-phosphate isomerase) | 0.01 | 0.84 | -0.26 | 0.41 | 1.00 | 0.00 |
| Glycolysis / Gluconeogenesis | 2,3-bisphosphoglycerate-dependent phosphoglycerate | 0.03 | 0.96 | -0.06 | 0.87 | 0.91 | -0.14 |
| Glycolysis / Gluconeogenesis | L-2-hydroxyisocaproate dehydrogenase (EC 1.1.1.-) | 0.01 | 0.69 | -0.54 | 0.08 | 0.59 | -0.75 |
| Glycolysis / Gluconeogenesis | Triosephosphate isomerase (TIM) (EC 5.3.1.1) (Triose-phosphate isomerase) | 0.01 | 0.84 | -0.26 | 0.41 | 1.00 | 0.00 |
| Glycolysis / Gluconeogenesis | Predicted oxidoreductase | 0.01 | 0.45 | -1.15 | 0.37 | 1.16 | 0.21 |
| Glycolysis / Gluconeogenesis | Phosphoenolpyruvate carboxykinase (ATP) | 0.04 | 2.07 | 1.05 | 0.22 | 1.27 | 0.34 |
| Glycolysis / Gluconeogenesis | Phosphoenolpyruvate carboxykinase (ATP) (EC 4.1.1.49) | 0.04 | 2.07 | 1.05 | 0.22 | 1.27 | 0.34 |

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|---|---|---|---|---|---|---|---|
| Glycolysis / Gluconeogenesis | Phosphoenolpyruvate carboxykinase (ATP) | 0.04 | 2.07 | 1.05 | 0.22 | 1.27 | 0.34 |
| Glycosyltransferases | 3-oxoacyl-[acyl-carrier-protein] synthase 3 | 0.03 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| GTP-binding proteins | GTP-binding protein-BipA-EF-TU family | 0.04 | 0.78 | -0.37 | 0.01 | 0.22 | -2.20 |
| GTP-binding proteins | GTPase obg (GTP-binding protein obg) | 0.03 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| GTP-binding proteins | GTPase obg (GTP-binding protein obg) | 0.03 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Heat shock proteins | 60 kDa heat shock protein (Fragment) | 0.78 | 1.23 | 0.30 | 0.01 | 0.48 | -1.06 |
| Heat shock proteins | 60 kDa heat shock protein (HSP60) (Fragment) | 0.67 | 1.21 | 0.28 | 0.01 | 0.50 | -1.01 |
| Histidine metabolism | rRNA methylase | 0.01 | 2.60 | 1.38 | 0.17 | 1.53 | 0.62 |
| Histidine metabolism | 23S rRNA methyltransferase (EC 2.1.1.-) | 0.01 | 2.60 | 1.38 | 0.17 | 1.53 | 0.62 |
| Homologous recombination | DNA topoisomerase I (EC 5.99.1.2) | 0.00 | 1.15 | 0.20 | 0.05 | 0.56 | -0.85 |
| Homologous recombination | DNA topoisomerase I (EC 5.99.1.2) | 0.00 | 1.15 | 0.20 | 0.05 | 0.56 | -0.85 |
| Homologous recombination | DNA topoisomerase I, TopA | 0.00 | 1.15 | 0.20 | 0.05 | 0.56 | -0.85 |
| Lysine biosynthesis | UDP-N-acetylmuramyl-tripeptide synthetase (UDP-MurNAc-tripeptide synthetase) (EC 6.3.2.-) | 0.72 | 1.37 | 0.46 | 0.00 | 0.35 | -1.52 |
| Lysine biosynthesis | UDP-N-acetylmuramyl tripeptide synthase | 0.87 | 1.28 | 0.36 | 0.00 | 0.35 | -1.52 |
| Lysine biosynthesis | UDP-N-acetylmuramyl-tripeptide synthetase | 0.01 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Lysine degradation | Predicted dehydrogenase related protein | 0.25 | 0.72 | -0.47 | 0.03 | 0.35 | -1.52 |
| Lysine degradation | Predicted dehydrogenase related protein | 0.25 | 0.72 | -0.47 | 0.03 | 0.35 | -1.52 |
| Membrane protease | Membrane protease subunit, stomatin/prohibitin family | 0.00 | 3.93 | 1.98 | 0.02 | 2.07 | 1.05 |
| Metabolism of Other Amino Acids | Dihydroorotate dehydrogenase A (fumarate) | 0.00 | 0.43 | -1.22 | 0.01 | 0.43 | -1.22 |
| Metabolism of Other Amino Acids | Dihydroorotase (EC 3.5.2.3) | 0.00 | 0.35 | -1.52 | 0.00 | 0.35 | -1.52 |
| Metabolism of Other Amino Acids | Dihydroorotate dehydrogenase | 0.00 | 0.35 | -1.52 | 0.00 | 0.35 | -1.52 |
| Metabolism of Other Amino Acids | Dihydroorotate dehydrogenase | 0.00 | 0.35 | -1.52 | 0.00 | 0.35 | -1.52 |

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| Metabolism of Other Amino Acids | Dihydroorotate oxidase B, catalytic subunit (EC 1.3.3.1) | 0.03 | 2.07 | 1.05 | 1.00 | 1.00 | 0.00 |
| Metabolism of Other Amino Acids | Dihydroorotate dehydrogenase A | 0.03 | 2.07 | 1.05 | 1.00 | 1.00 | 0.00 |
| Mismatch repair | Predicted N6-adenine-specific DNA methylase | 0.03 | 2.33 | 1.22 | 0.22 | 1.27 | 0.34 |
| Mismatch repair | Predicted N6-adenine-specific DNA methylase | 0.03 | 2.33 | 1.22 | 0.22 | 1.27 | 0.34 |
| Nicotinate and nicotinamide metabolism | Amidophosphoribosyltransferase (ATase) (EC 2.4.2.14) | 0.00 | 0.27 | -1.87 | 0.00 | 0.27 | -1.87 |
| Nicotinate and nicotinamide metabolism | Orotate phosphoribosyltransferase (OPRT) (OPRTase) (EC 2.4.2.10) | 0.02 | 0.48 | -1.05 | 0.03 | 0.48 | -1.05 |
| Nucleotide excision repair | UPF0042 nucleotide-binding protein LCABL_10730 | 0.61 | 1.00 | 0.00 | 0.00 | 0.35 | -1.52 |
| Nucleotide excision repair | UPF0042 nucleotide-binding protein LSEI_0959 | 0.61 | 1.00 | 0.00 | 0.00 | 0.35 | -1.52 |
| One carbon pool by folate | Formate--tetrahydrofolate ligase (EC 6.3.4.3) | 0.01 | 0.56 | -0.84 | 0.00 | 0.34 | -1.56 |
| One carbon pool by folate | Formate--tetrahydrofolate ligase (EC 6.3.4.3) | 0.01 | 0.60 | -0.74 | 0.00 | 0.31 | -1.69 |
| One carbon pool by folate | Uncharacterized protein | 0.01 | 0.56 | -0.84 | 0.00 | 0.34 | -1.56 |
| Oxidative phosphorylation | Septum formation-inhibiting ATPase | 0.03 | 2.07 | 1.05 | 1.00 | 1.00 | 0.00 |
| Oxidative phosphorylation | ATP synthase subunit alpha (EC 3.6.3.14) | 0.01 | 0.69 | -0.53 | 0.03 | 0.54 | -0.89 |
| Oxidative phosphorylation | ATP synthase subunit beta (EC 3.6.3.14) | 0.02 | 0.92 | -0.12 | 0.19 | 0.72 | -0.47 |
| Oxidative phosphorylation | ATP synthase subunit alpha (EC 3.6.3.14) | 0.01 | 0.69 | -0.53 | 0.03 | 0.54 | -0.89 |
| Oxidative phosphorylation | ATP synthase gamma chain | 0.00 | 0.41 | -1.27 | 0.00 | 0.31 | -1.71 |
| Oxidative phosphorylation | ATP synthase gamma chain | 0.00 | 0.41 | -1.27 | 0.00 | 0.31 | -1.71 |
| Oxidative phosphorylation | NADH oxidase (EC 1.6.99.3) | 0.65 | 1.13 | 0.17 | 0.02 | 1.59 | 0.67 |
| Oxidative phosphorylation | Putative NADH-flavin reductase | 0.10 | 0.66 | -0.60 | 0.00 | 0.32 | -1.65 |
| Oxidative phosphorylation | ATP synthase subunit delta | 0.13 | 0.66 | -0.61 | 0.01 | 0.43 | -1.22 |
| Oxidative phosphorylation | ATP synthase subunit delta (ATP synthase | 0.13 | 0.66 | -0.61 | 0.01 | 0.43 | -1.22 |
| Oxidative phosphorylation | ATP synthase subunit beta (EC 3.6.3.14) | 0.02 | 0.92 | -0.12 | 0.19 | 0.72 | -0.47 |
| Oxidative phosphorylation | ATP synthase subunit beta (EC 3.6.3.14) | 0.02 | 0.85 | -0.23 | 0.25 | 0.72 | -0.47 |
| Oxidative phosphorylation | F1F0-ATPase subunit beta | 0.02 | 0.92 | -0.12 | 0.19 | 0.72 | -0.47 |

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| Pantothenate and CoA biosynthesis | 2-dehydropantoate 2-reductase (EC 1.1.1.169) | 1.00 | 1.00 | 0.00 | 0.01 | 2.87 | 1.52 |
| Pentose phosphate pathway | 6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44) | 0.00 | 0.88 | -0.19 | 0.28 | 1.01 | 0.01 |
| Peptidoglycan biosynthesis | FemAB family protein | 0.18 | 0.74 | -0.43 | 0.02 | 0.40 | -1.31 |
| Phenylalanine metabolism | Predicted iron-dependent peroxidase | 0.05 | 0.62 | -0.70 | 0.40 | 1.10 | 0.14 |
| Phenylalanine metabolism | Predicted iron-dependent peroxidase | 0.05 | 0.62 | -0.70 | 0.40 | 1.10 | 0.14 |
| Phenylalanine metabolism | Alkyl hydroperoxide reductase, C subunit (EC 1.11.1.15) | 0.00 | 0.32 | -1.65 | 0.76 | 0.83 | -0.27 |
| Phosphotransferase system | Cellobiose-specific PTS system IIC component | 0.47 | 1.56 | 0.64 | 0.00 | 0.35 | -1.52 |
| Phosphotransferase system | Beta-glucoside bgl operon antiterminator | 0.53 | 1.46 | 0.55 | 0.00 | 0.10 | -3.29 |
| Phosphotransferase system | Transcription antiterminator LacT | 0.44 | 1.50 | 0.59 | 0.00 | 0.10 | -3.33 |
| Phosphotransferase system | Fructose/mannose phosphotransferase system IIB component | 0.07 | 0.82 | -0.28 | 0.00 | 0.11 | -3.17 |
| Phosphotransferase system | Phosphotransferase system, galactitol-specific IIB component | 0.80 | 1.35 | 0.43 | 0.00 | 0.16 | -2.60 |
| Phosphotransferase system | Phosphotransferase system sugar-specific EII component | 0.23 | 1.82 | 0.86 | 0.03 | 0.38 | -1.38 |
| Phosphotransferase system | Phosphotransferase system galacitol-specific IIA domain (Ntr-type) | 0.30 | 1.72 | 0.78 | 0.03 | 0.38 | -1.38 |
| Porphyrin and chlorophyll metabolism | Glutamyl-tRNA synthetase (EC 6.1.1.17) (Glutamate--tRNA ligase) (GluRS) | 0.68 | 1.17 | 0.23 | 0.01 | 0.42 | -1.24 |
| Porphyrin and chlorophyll metabolism | Glutamyl-tRNA synthetase (EC 6.1.1.17) (Glutamate--tRNA ligase) (GluRS) | 0.68 | 1.17 | 0.23 | 0.01 | 0.42 | -1.24 |
| Predicted membrane protein | Predicted membrane protein | 0.50 | 1.23 | 0.30 | 0.00 | 0.54 | -0.88 |
| Propanoate metabolism | Pyruvate/oxaloacetate carboxyltransferase | 0.00 | 18.89 | 4.24 | 0.00 | 33.09 | 5.05 |
| Propanoate metabolism | Pyruvate/oxaloacetate carboxyltransferase | 0.00 | 18.89 | 4.24 | 0.00 | 33.09 | 5.05 |
| protein degradation | Peptidase M3B, oligoendopeptidase F | 0.03 | 0.72 | -0.47 | 0.50 | 0.76 | -0.40 |
| protein degradation | Oligoendopeptidase F2 (EC 3.4.24.-) | 0.05 | 0.92 | -0.11 | 0.37 | 0.76 | -0.40 |

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| protein degradation | Oligoendopeptidase F | 0.03 | 0.72 | -0.47 | 0.50 | 0.76 | -0.40 |
| protein degradation | Aminopeptidase S (EC 3.4.11.-) | 0.00 | 4.47 | 2.16 | 0.02 | 2.07 | 1.05 |
| protein degradation | Peptidase T | 0.00 | 0.32 | -1.65 | 0.00 | 0.32 | -1.65 |
| protein degradation | Peptidase T | 0.00 | 0.32 | -1.65 | 0.00 | 0.32 | -1.65 |
| protein degradation | Cysteine aminopeptidase C2 (Bleomycin hydrolase) (EC 3.4.22.40) | 0.18 | 1.07 | 0.10 | 0.02 | 0.60 | -0.74 |
| protein degradation | Leucyl aminopeptidase (Aminopeptidase T) | 0.03 | 2.74 | 1.45 | 0.20 | 0.65 | -0.62 |
| protein synthesis | Uncharacterized protein | 0.00 | 1.99 | 0.99 | 0.00 | 1.47 | 0.55 |
| protein synthesis | Elongation factor Tu (EF-Tu) | 0.00 | 1.99 | 0.99 | 0.00 | 1.47 | 0.55 |
| protein synthesis | Putative elongation factor Tu (Fragment) | 0.00 | 2.02 | 1.01 | 0.00 | 1.51 | 0.59 |
| protein synthesis | Putative elongation factor Tu (Fragment) | 0.00 | 1.95 | 0.97 | 0.00 | 1.44 | 0.53 |
| protein synthesis | Putative elongation factor Tu (Fragment) | 0.00 | 2.02 | 1.01 | 0.00 | 1.51 | 0.59 |
| protein synthesis | Oligopeptidase F, Metallo peptidase, MEROPS family M03B | 0.03 | 0.72 | -0.47 | 0.50 | 0.76 | -0.40 |
| Purine metabolism | Adenylosuccinate lyase (EC 4.3.2.2) | 0.02 | 0.75 | -0.42 | 0.00 | 0.41 | -1.30 |
| Purine metabolism | Inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205) | 0.00 | 0.29 | -1.79 | 0.00 | 0.21 | -2.22 |
| Purine metabolism | GMP reductase (EC 1.7.1.7) | 0.02 | 0.45 | -1.15 | 0.00 | 0.29 | -1.77 |
| Purine metabolism | 5'-nucleotidase/2',3'-cyclic phosphodiesterase related esterase | 0.10 | 0.66 | -0.60 | 0.00 | 0.32 | -1.65 |
| Purine metabolism | 2 ,3-cyclic-nucleotide 2-phosphodiesterase (EC 3.1.4.16) | 0.06 | 0.61 | -0.72 | 0.00 | 0.29 | -1.77 |
| Purine metabolism | 5'-nucleotidase/2',3'-cyclic phosphodiesterase related esterase | 0.10 | 0.66 | -0.60 | 0.00 | 0.32 | -1.65 |
| Purine metabolism | Hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) | 0.00 | 4.47 | 2.16 | 1.00 | 1.00 | 0.00 |
| Purine metabolism | Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) | 0.00 | 3.67 | 1.87 | 1.00 | 1.00 | 0.00 |
| Purine metabolism | Apo-citrate lyase phosphoribosyl-dephospho-CoA transferase (EC 2.7.7.61) | 0.03 | 4.73 | 2.24 | 1.00 | 1.80 | 0.85 |

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| Purine metabolism | Adenylosuccinate lyase (EC 4.3.2.2) | 0.02 | 0.75 | -0.42 | 0.00 | 0.41 | -1.30 |
| Purine metabolism | Adenylosuccinate synthetase (AMPSase) | 0.00 | 0.28 | -1.84 | 0.00 | 0.13 | -2.95 |
| Purine metabolism | Adenylosuccinate synthetase | 0.00 | 0.28 | -1.84 | 0.00 | 0.13 | -2.95 |
| Purine metabolism | Xanthine phosphoribosyltransferase (XPRTase) (EC 2.4.2.22) | 0.04 | 0.71 | -0.50 | 0.04 | 0.41 | -1.28 |
| Purine metabolism | Xanthine phosphoribosyltransferase (XPRTase) (EC 2.4.2.22) | 0.04 | 0.71 | -0.50 | 0.04 | 0.41 | -1.28 |
| Purine metabolism | Xanthine phosphoribosyltransferase (XPRTase) (EC 2.4.2.22) | 0.04 | 0.71 | -0.50 | 0.04 | 0.41 | -1.28 |
| Purine metabolism | Phosphoribosylamine--glycine ligase (EC 6.3.4.13) | 0.00 | 0.13 | -2.99 | 0.00 | 0.13 | -2.99 |
| Purine metabolism | Bifunctional purine biosynthesis protein PurH | 0.00 | 0.08 | -3.58 | 0.00 | 0.08 | -3.58 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase | 0.00 | 0.22 | -2.16 | 0.00 | 0.22 | -2.16 |
| Purine metabolism | Ribonucleoside-diphosphate reductase, beta chain (EC 1.17.4.1) | 0.00 | 0.43 | -1.22 | 0.01 | 0.43 | -1.22 |
| Purine metabolism | Phosphoribosylamine--glycine ligase (GARS) | 0.00 | 0.10 | -3.29 | 0.00 | 0.10 | -3.29 |
| Purine metabolism | Bifunctional purine biosynthesis protein PurH | 0.00 | 0.07 | -3.79 | 0.00 | 0.07 | -3.79 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase | 0.00 | 0.19 | -2.40 | 0.00 | 0.19 | -2.40 |
| Purine metabolism | Ribonucleotide reductase, beta subunit | 0.00 | 0.43 | -1.22 | 0.01 | 0.43 | -1.22 |
| Purine metabolism | Phosphoribosylamine-glycine ligase | 0.00 | 0.13 | -2.94 | 0.00 | 0.13 | -2.94 |
| Purine metabolism | Bifunctional purine biosynthesis protein PurH | 0.00 | 0.08 | -3.73 | 0.00 | 0.08 | -3.73 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6) | 0.00 | 0.20 | -2.32 | 0.00 | 0.20 | -2.32 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6) | 0.00 | 0.19 | -2.40 | 0.00 | 0.19 | -2.40 |

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| Purine metabolism | Phosphoribosylformylglycinamidine (FGAM) synthase, PurS component | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | 5-(Carboxyamino)imidazole ribonucleotide synthase (EC 4.1.1.21) | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | Conserved purine biosynthesis cluster protein | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | Phosphoribosylaminoimidazole carboxylase, ATPase subunit (EC 4.1.1.21) | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | Phosphoribosylformylglycinamidine (FGAM) synthase, PurS component | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | Phosphoribosylaminoimidazole carboxylase (NCAIR synthetase) | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | Phosphoribosylformylglycinamidine (Fgam) synthase, purs component | 0.00 | 0.38 | -1.38 | 0.01 | 0.38 | -1.38 |
| Purine metabolism | Phosphoribosylformylglycinamidine cyclo-ligase (EC 6.3.3.1) | 0.02 | 0.43 | -1.22 | 0.03 | 0.43 | -1.22 |
| Purine metabolism | Phosphoribosylformylglycinamidine cyclo-ligase (EC 6.3.3.1) | 0.02 | 0.43 | -1.22 | 0.03 | 0.43 | -1.22 |
| Purine metabolism | N5-carboxyaminoimidazole ribonucleotide mutase (N5-CAIR mutase) (EC 5.4.99.18) | 0.02 | 0.48 | -1.05 | 0.03 | 0.48 | -1.05 |
| Purine metabolism | Adenylate kinase (AK) (EC 2.7.4.3) (ATP-AMP transphosphorylase) | 0.22 | 1.80 | 0.85 | 0.01 | 0.35 | -1.53 |
| Purine metabolism | Adenylate kinase (AK) (EC 2.7.4.3) (ATP-AMP transphosphorylase) | 0.14 | 1.94 | 0.96 | 0.01 | 0.37 | -1.42 |
| Purine metabolism | Ribonucleoside-diphosphate reductase (EC 1.17.4.1) | 0.19 | 0.87 | -0.20 | 0.00 | 0.16 | -2.60 |
| Purine metabolism | Ribonucleoside-diphosphate reductase (EC 1.17.4.1) | 0.19 | 0.87 | -0.20 | 0.00 | 0.16 | -2.60 |
| Purine metabolism | Ribonucleoside-diphosphate reductase (EC 1.17.4.1) | 0.19 | 0.87 | -0.20 | 0.00 | 0.16 | -2.60 |
| Purine metabolism | Phospho ribosylaminoimidazole carboxylase (AIR carboxylase), ATPase subunit (EC 4.1.1.21) | 0.20 | 1.53 | 0.62 | 0.03 | 1.80 | 0.85 |

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| Purine metabolism | Adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7) | 0.02 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Purine metabolism | Adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7) | 0.02 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| Purine metabolism | Phosphoribosylformylglycinamidine synthase 2 (EC 6.3.5.3) | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |
| Purine metabolism | Phosphoribosylformylglycinamidine synthase 2 (EC 6.3.5.3) | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |
| Purine metabolism | Phosphoribosylformylglycinamidine synthase 2 (EC 6.3.5.3) | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |
| Purine metabolism | Phosphoribosylformylglycinamidine synthase 2 (EC 6.3.5.3) | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |
| Purine metabolism | Phosphoribosylformylglycinamidine synthase 2 (EC 6.3.5.3) | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |
| Pyrimidine metabolism | Bifunctional protein pyrR | 0.00 | 0.34 | -1.56 | 0.00 | 0.17 | -2.60 |
| Pyrimidine metabolism | Aspartate carbamoyltransferase (EC 2.1.3.2) | 0.05 | 0.53 | -0.92 | 0.00 | 0.29 | -1.77 |
| Pyrimidine metabolism | Aspartate carbamoyltransferase (EC 2.1.3.2) (Aspartate transcarbamylase) | 0.05 | 0.53 | -0.92 | 0.00 | 0.29 | -1.77 |
| Pyrimidine metabolism | Dihydroorotase (EC 3.5.2.3) | 0.00 | 0.29 | -1.77 | 0.00 | 0.29 | -1.77 |
| Pyrimidine metabolism | Thioredoxin | 0.19 | 0.79 | -0.34 | 0.02 | 2.26 | 1.18 |
| Pyrimidine metabolism | Aspartate carbamoyltransferase (EC 2.1.3.2) | 0.09 | 0.63 | -0.67 | 0.00 | 0.35 | -1.52 |
| Pyrimidine metabolism | Aspartate carbamoyltransferase (EC 2.1.3.2) (Aspartate transcarbamylase) | 0.13 | 0.66 | -0.61 | 0.01 | 0.43 | -1.22 |
| Pyrimidine metabolism | Uncharacterized protein | 0.09 | 0.63 | -0.67 | 0.00 | 0.35 | -1.52 |
| Pyrimidine metabolism | Cytidylate kinase (CK) (EC 2.7.4.14) (Cytidine monophosphate kinase) | 0.03 | 2.33 | 1.22 | 0.17 | 1.53 | 0.62 |
| Pyrimidine metabolism | Carbamoyl-phosphate synthase large chain (EC 6.3.5.5) | 0.00 | 0.24 | -2.05 | 0.00 | 0.08 | -3.70 |
| Pyrimidine metabolism | Uridylate kinase (UK) (Uridine monophosphate kinase) | 0.92 | 1.23 | 0.30 | 0.00 | 0.22 | -2.20 |
| Pyrimidine metabolism | Uridylate kinase (UK) (EC 2.7.4.22) | 0.92 | 1.23 | 0.30 | 0.00 | 0.22 | -2.20 |
| Pyrimidine metabolism | Orotidine 5'-phosphate decarboxylase (EC 4.1.1.23) (OMP decarboxylase) | 0.03 | 0.56 | -0.85 | 0.05 | 0.56 | -0.85 |

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| Pyruvate metabolism | Acetate kinase 1 (EC 2.7.2.1) (Acetokinase 1) | 0.01 | 3.90 | 1.96 | 0.00 | 2.96 | 1.57 |
| Pyruvate metabolism | Bifunctional enzyme: alcohol dehydrogenase | 0.00 | 2.28 | 1.19 | 0.00 | 0.45 | -1.16 |
| Pyruvate metabolism | Pyruvate carboxylase | 0.03 | 2.07 | 1.05 | 0.01 | 2.33 | 1.22 |
| Pyruvate metabolism | Predicted acetyltransferase | 0.03 | 3.67 | 1.87 | 0.00 | 4.20 | 2.07 |
| Pyruvate metabolism | Pyruvate phosphate dikinase (EC 2.7.9.1) | 0.43 | 1.06 | 0.09 | 0.00 | 5.33 | 2.41 |
| Pyruvate metabolism | Phosphate acetyltransferase (Phosphotransacetylase) (EC 2.3.1.8) | 0.06 | 1.79 | 0.84 | 0.01 | 1.41 | 0.50 |
| Pyruvate metabolism | Phosphoenolpyruvate synthase/pyruvate phosphate dikinase | 0.50 | 1.09 | 0.13 | 0.00 | 5.78 | 2.53 |
| Pyruvate metabolism | Acetate kinase (EC 2.7.2.1) (Acetokinase) | 0.01 | 3.90 | 1.96 | 0.00 | 2.96 | 1.57 |
| Pyruvate metabolism | Uncharacterized protein | 0.01 | 3.90 | 1.96 | 0.00 | 2.96 | 1.57 |
| Pyruvate metabolism | Uncharacterized NAD(FAD)-dependent dehydrogenase | 0.00 | 0.41 | -1.29 | 0.00 | 0.29 | -1.77 |
| Pyruvate metabolism | Phosphotransacetylase (EC 2.3.1.8) | 0.03 | 1.89 | 0.92 | 0.04 | 1.30 | 0.37 |
| Pyruvate metabolism | Pyruvate kinase (EC 2.7.1.40) | 0.00 | 0.84 | -0.25 | 0.00 | 0.68 | -0.55 |
| Pyruvate metabolism | Formate C-acetyltransferase | 0.01 | 2.59 | 1.37 | 0.00 | 2.82 | 1.49 |
| Pyruvate metabolism | Pyruvate-formate lyase-activating enzyme | 0.03 | 3.67 | 1.87 | 0.00 | 7.14 | 2.84 |
| Pyruvate metabolism | Formate acetyltransferase activating enzyme (EC 1.97.1.4) | 0.03 | 3.67 | 1.87 | 0.00 | 7.14 | 2.84 |
| Pyruvate metabolism | Acetyltransferase (EC 2.3.1.-) | 0.03 | 3.67 | 1.87 | 0.00 | 4.20 | 2.07 |
| Pyruvate metabolism | Pyruvate-formate lyase-activating enzyme | 0.03 | 3.67 | 1.87 | 0.00 | 6.07 | 2.60 |
| Pyruvate metabolism | Acetoin/pyruvate dehydrogenase complex | 0.06 | 2.52 | 1.33 | 0.02 | 1.64 | 0.72 |
| Pyruvate metabolism | Pyruvate phosphate dikinase | 0.55 | 1.12 | 0.16 | 0.00 | 5.97 | 2.58 |
| Pyruvate metabolism | Phosphotransacetylase | 0.05 | 1.81 | 0.85 | 0.01 | 1.41 | 0.50 |
| Pyruvate metabolism | Acetoin/pyruvate dehydrogenase complex | 0.06 | 2.52 | 1.33 | 0.02 | 1.64 | 0.72 |
| Pyruvate metabolism | Pyruvate, phosphate dikinase | 0.55 | 1.12 | 0.16 | 0.00 | 5.97 | 2.58 |
| Pyruvate metabolism | Uncharacterized NAD(FAD)-dependent dehydrogenase | 0.70 | 1.14 | 0.19 | 0.02 | 1.64 | 0.72 |
| RNA degradation | Chaperone protein DnaK (HSP70) | 0.90 | 1.29 | 0.37 | 0.01 | 0.62 | -0.70 |

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|---|---|---|---|---|---|---|---|
| RNA degradation | Chaperone protein DnaK (HSP70) | 0.85 | 1.28 | 0.36 | 0.01 | 0.60 | -0.73 |
| RNA degradation | 60 kDa chaperonin (GroEL protein) (Protein Cpn60) | 0.86 | 1.30 | 0.37 | 0.00 | 0.43 | -1.22 |
| RNA degradation | 60 kDa chaperonin (GroEL protein) (Protein Cpn60) | 0.86 | 1.30 | 0.37 | 0.00 | 0.43 | -1.22 |
| RNA degradation | GroEL (Fragment) | 0.55 | 1.17 | 0.23 | 0.00 | 0.47 | -1.08 |
| RNA degradation | GroEL (Fragment) | 0.67 | 1.21 | 0.28 | 0.01 | 0.50 | -1.01 |
| RNA degradation | GroEL (Fragment) | 0.55 | 1.17 | 0.23 | 0.00 | 0.47 | -1.08 |
| RNA transport | Translation initiation factor IF-1 | 0.03 | 2.33 | 1.22 | 1.00 | 1.00 | 0.00 |
| RNA transport | Translation initiation factor IF-1 | 0.03 | 2.33 | 1.22 | 1.00 | 1.00 | 0.00 |
| Signal Transduction | Protein tyrosine phosphatase | 0.01 | 3.11 | 1.63 | 0.26 | 0.79 | -0.34 |
| Signal Transduction | Protein-tyrosine phosphatase (Putative) (EC 3.1.3.48) | 0.01 | 3.11 | 1.63 | 0.26 | 0.79 | -0.34 |
| Starch and sucrose metabolism | ADP-glucose pyrophosphorylase | 0.00 | 3.67 | 1.87 | 0.00 | 3.93 | 1.98 |
| Starch and sucrose metabolism | Glucose-1-phosphate adenylyltransferase, subunit (EC 2.7.7.27) | 0.00 | 3.67 | 1.87 | 0.00 | 3.93 | 1.98 |
| Starch and sucrose metabolism | ADP-glucose pyrophosphorylase | 0.00 | 3.67 | 1.87 | 0.00 | 3.93 | 1.98 |
| Starch and sucrose metabolism | Glucose-1-phosphate adenylyltransferase (EC 2.7.7.27) | 0.00 | 2.87 | 1.52 | 0.00 | 3.13 | 1.65 |
| Starch and sucrose metabolism | Maltosephosphorylase | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Starch and sucrose metabolism | Maltose phosphorylase (EC 2.4.1.8) | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Starch and sucrose metabolism | Trehalose-6-phosphate hydrolase | 0.03 | 0.75 | -0.42 | 0.12 | 1.25 | 0.32 |
| Streptomycin biosynthesis | dTDP-4-dehydrorhamnose 3,5-epimerase | 0.40 | 0.90 | -0.16 | 0.03 | 0.38 | -1.38 |
| Streptomycin biosynthesis | dTDP-4-dehydrorhamnose 3,5-epimerase related enzyme | 0.40 | 0.90 | -0.16 | 0.03 | 0.38 | -1.38 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ chaperones | 0.04 | 0.65 | -0.62 | 0.00 | 0.12 | -3.09 |
| Stress protein | ATP-dependent clp protease ATP-binding subunit | 0.04 | 0.66 | -0.60 | 0.00 | 0.12 | -3.06 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ chaperones | 0.04 | 0.67 | -0.59 | 0.00 | 0.11 | -3.15 |
| stress protein | Similar to universal stress protein, UspA family | 0.00 | 0.14 | -2.86 | 0.00 | 0.40 | -1.32 |
| Stress protein | Chaperone ClpB | 0.01 | 0.42 | -1.25 | 0.00 | 0.21 | -2.27 |

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|---|---|---|---|---|---|---|---|
| Stress protein | Chaperone ClpB | 0.01 | 0.42 | -1.25 | 0.00 | 0.21 | -2.27 |
| Stress protein | Membrane protease subunit, stomatin/prohibitin family | 0.00 | 5.00 | 2.32 | 0.02 | 2.33 | 1.22 |
| stress protein | Similar to universal stress protein, UspA family | 0.76 | 1.15 | 0.20 | 0.01 | 0.39 | -1.35 |
| Stress protein | ATP-dependent protease HslVU (ClpYQ), ATPase subunit | 0.49 | 1.53 | 0.61 | 0.03 | 0.38 | -1.38 |
| Stress protein | ATP-dependent Hsl protease, ATP-binding subunit HslU | 0.49 | 1.53 | 0.61 | 0.03 | 0.38 | -1.38 |
| Stress protein | ATP-dependent protease HslVU (ClpYQ), ATPase subunit | 0.49 | 1.53 | 0.61 | 0.03 | 0.38 | -1.38 |
| Stress protein | ATP-dependent Clp protease ATP-binding subunit ClpX | 0.69 | 1.41 | 0.49 | 0.00 | 0.29 | -1.78 |
| Stress protein | ATPase with chaperone activity, ATP-binding subunit | 0.05 | 0.67 | -0.57 | 1.00 | 0.11 | -3.13 |
| Terpenoid backbone biosynthesis | Hydroxymethylglutaryl-CoA reductase | 1.00 | 1.00 | 0.00 | 0.03 | 2.07 | 1.05 |
| Terpenoid backbone biosynthesis | Hydroxymethylglutaryl-CoA reductase (EC 1.1.1.88) | 1.00 | 1.00 | 0.00 | 0.03 | 2.07 | 1.05 |
| Transcription | Transcription antitermination protein nusG | 0.85 | 1.18 | 0.24 | 0.05 | 0.46 | -1.11 |
| Transcription factors | Transcriptional regulator | 0.36 | 0.79 | -0.33 | 0.03 | 0.38 | -1.38 |
| Translation | Sex pheromone staph-cAM373 | 0.04 | 2.07 | 1.05 | 1.00 | 1.00 | 0.00 |
| Translation Factors | Tuf (Fragment) | 0.00 | 1.99 | 0.99 | 0.00 | 1.44 | 0.52 |
| Translation Factors | Elongation factor Tu (Fragment) | 0.00 | 1.97 | 0.98 | 0.00 | 1.46 | 0.54 |
| Translation Factors | Elongation factor Tu (Fragment) | 0.00 | 1.97 | 0.98 | 0.00 | 1.46 | 0.54 |
| Translation Factors | Tuf (Fragment) | 0.00 | 1.97 | 0.98 | 0.00 | 1.46 | 0.54 |
| transport | ABC-type uncharacterized transport system, periplasmic component | 0.01 | 0.56 | -0.84 | 0.01 | 0.37 | -1.43 |
| transport | Maltose ABC transporter substrate binding protein | 0.02 | 2.33 | 1.22 | 0.00 | 3.39 | 1.76 |
| transport | ABC-type uncharacterized transport system, periplasmic component | 0.01 | 0.53 | -0.91 | 0.00 | 0.35 | -1.50 |
| transport | Carbohydrate ABC transporter | 0.00 | 4.73 | 2.24 | 0.00 | 4.47 | 2.16 |
| transport | ABC transporter, permease protein | 0.00 | 4.73 | 2.24 | 0.00 | 4.47 | 2.16 |
| transport | ABC-type proline/glycine betaine transport system, ATPase component | 0.04 | 3.67 | 1.87 | 0.00 | 4.20 | 2.07 |
| transport | Glycine betaine/carnitine | 0.04 | 3.67 | 1.87 | 0.00 | 4.20 | 2.07 |

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|---|---|---|---|---|---|---|---|
| transport | Cell-division associated ABC transporter, ATP binding FtsE subunit | 0.00 | 3.93 | 1.98 | 0.03 | 2.33 | 1.22 |
| transport | Putative ATP binding protein | 0.01 | 0.46 | -1.13 | 0.00 | 0.25 | -1.98 |
| transport | ABC transporter protein | 0.01 | 0.46 | -1.13 | 0.00 | 0.25 | -1.98 |
| transport | ABC-type oligopeptide transport system, periplasmic component | 0.18 | 1.00 | 0.00 | 0.00 | 0.07 | -3.76 |
| transport | ABC transporter, oligopeptide-binding protein | 0.16 | 1.02 | 0.02 | 0.00 | 0.06 | -4.02 |
| transport | ABC-type oligopeptide transport system | 0.16 | 0.98 | -0.03 | 0.00 | 0.08 | -3.67 |
| transport | Multiple sugar ABC transporter | 0.21 | 2.33 | 1.22 | 0.00 | 5.27 | 2.40 |
| transport | Multiple sugar ABC transporter ATPase component | 0.21 | 2.07 | 1.05 | 0.00 | 4.73 | 2.24 |
| transport | ABC-type sugar transport system, ATPase | 0.04 | 3.09 | 1.63 | 0.20 | 0.65 | -0.62 |
| transport | Carbohydrate ABC transporter ATP-binding protein, CUT1 family | 0.04 | 2.91 | 1.54 | 0.20 | 0.65 | -0.62 |
| transport | Glutamine transport ATP-binding protein | 0.05 | 2.87 | 1.52 | 1.00 | 1.00 | 0.00 |
| transport | Amino acid ABC transporter ATP-binding protein, PAAT family | 0.05 | 2.87 | 1.52 | 1.00 | 1.00 | 0.00 |
| transport | ABC transporter related | 0.00 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| transport | ABC-type antimicrobial peptide transport system | 0.00 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| transport | Amino acid ABC transporter substrate-binding protein | 0.04 | 2.60 | 1.38 | 1.00 | 1.00 | 0.00 |
| transport | Extracellular solute-binding protein, family 1 | 0.00 | 4.73 | 2.24 | 0.00 | 4.47 | 2.16 |

Table A2: Proteomics data of *Lactobacillus casei* GCRL163with in mMRS in absent of Tween 80 with three levels of lactose (0%, 0.2% or 1%).

**NTW = No Tween 80**
**1% = 1% Lactose**
**0.2% = 0.2% Lactose**
**0% = 0% Lactose**

| Pathway | Protein Name | *P*-Value NTW 0.2 vs 1 | Fold Change | Log 2 Fold change | *P*-Value NTW 0 vs 1 | Fold Change | Log 2 Fold change |
|---|---|---|---|---|---|---|---|
| ABC transporters | Extracellular solute-binding protein family 5 | 0.00 | 0.38 | -1.41 | 0.00 | 0.02 | -5.48 |
| ABC transporters | Extracellular solute-binding protein family 5 | 0.00 | 0.38 | -1.41 | 0.00 | 0.02 | -5.48 |
| ABC transporters | Cell division ATP-binding protein FtsE | 0.03 | 3.93 | 1.98 | 0.03 | 2.33 | 1.22 |
| ABC transporters | MalK1 | 0.17 | 2.33 | 1.22 | 0.03 | 2.87 | 1.52 |
| Alanine | Aspartate aminotransferase (EC 2.6.1.1) | 0.28 | 0.94 | -0.09 | 0.01 | 0.37 | -1.45 |
| Alanine, aspartate and glutamate metabolism | Glutamine synthetase (EC 6.3.1.2) | 0.00 | 2.07 | 1.05 | 0.00 | 1.81 | 0.86 |
| Alanine, aspartate and glutamate metabolism | Glutamine synthetase (EC 6.3.1.2) | 0.00 | 2.07 | 1.05 | 0.00 | 1.81 | 0.86 |
| Alanine, aspartate and glutamate metabolism | Glutamine synthetase (EC 6.3.1.2) | 0.00 | 2.15 | 1.10 | 0.00 | 1.87 | 0.90 |
| Alanine, aspartate and glutamate metabolism | Alanine dehydrogenase (EC 1.4.1.1) | 0.01 | 3.13 | 1.65 | 1.00 | 1.00 | 0.00 |
| Alanine, aspartate and glutamate metabolism | Cysteine sulfinate desulfinase/cysteine desulfurase related enzyme | 0.88 | 1.39 | 0.47 | 0.03 | 0.48 | -1.05 |
| Amino Acid Metabolism | Elongation factor 4 (EF-4) (EC 3.6.5.n1) (Ribosomal back-translocase LepA) | 0.06 | 1.00 | 0.00 | 0.04 | 0.56 | -0.85 |
| Amino sugar and nucleotide sugar metabolism | Phosphoglucosamine mutase (EC 5.4.2.10) | 0.01 | 1.96 | 0.97 | 0.01 | 2.07 | 1.05 |
| Amino sugar and nucleotide sugar metabolism | L-glutamine--D-fructose-6-phosphate amidotransferase (Glucosamine-6-phosphate | 0.04 | 1.41 | 0.49 | 0.00 | 3.39 | 1.76 |

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|---|---|---|---|---|---|---|---|
| Amino sugar and nucleotide sugar metabolism | Phosphoglucosamine mutase (EC 5.4.2.10) | 0.01 | 1.96 | 0.97 | 0.01 | 2.07 | 1.05 |
| Amino sugar and nucleotide sugar metabolism | NAD-dependent epimerase/dehydratase | 0.00 | 0.39 | -1.35 | 0.00 | 0.29 | -1.78 |
| Amino sugar and nucleotide sugar metabolism | YbfG | 0.17 | 3.67 | 1.87 | 0.00 | 3.40 | 1.77 |
| Amino sugar and nucleotide sugar metabolism | YbfG | 0.17 | 3.67 | 1.87 | 0.00 | 3.40 | 1.77 |
| Aminoacyl-tRNA biosynthesis | Phenylalanyl-tRNA synthetase alpha chain (EC 6.1.1.20) (Phenylalanine--tRNA ligase alpha | 0.03 | 1.53 | 0.62 | 0.00 | 2.60 | 1.38 |
| Aminoacyl-tRNA biosynthesis | S-adenosylmethionine:tRNA ribosyltransferase-isomerase (EC 5.-.-.-) (Queuosine biosynthesis protein QueA) | 0.00 | 0.64 | -0.64 | 0.00 | 0.22 | -2.16 |
| Aminoacyl-tRNA biosynthesis | Valyl-tRNA synthetase (EC 6.1.1.9) (Valine--tRNA ligase) | 0.00 | 0.10 | -3.37 | 0.01 | 0.41 | -1.30 |
| Aminoacyl-tRNA biosynthesis | Valyl-tRNA synthetase (EC 6.1.1.9) (Valine--tRNA ligase) (ValRS) | 0.00 | 0.16 | -2.66 | 0.04 | 0.49 | -1.02 |
| Aminoacyl-tRNA biosynthesis | Asparaginyl-tRNA synthetase (EC 6.1.1.22) (Asparagine--tRNA | 0.03 | 1.58 | 0.66 | 0.84 | 1.06 | 0.09 |
| Aminoacyl-tRNA biosynthesis | Aspartyl-tRNA synthetase (EC 6.1.1.12) (Aspartate--tRNA ligase) (AspRS) | 0.01 | 2.30 | 1.20 | 0.55 | 0.81 | -0.30 |
| Aminoacyl-tRNA biosynthesis | Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B (Asp/Glu-ADT subunit B) (EC | 0.26 | 1.26 | 0.34 | 0.03 | 1.52 | 0.61 |
| Aminoacyl-tRNA biosynthesis | Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B (Asp/Glu-ADT subunit B) (EC | 0.21 | 1.22 | 0.29 | 0.03 | 1.48 | 0.56 |
| Aminoacyl-tRNA biosynthesis | Translation initiation factor IF-2 | 0.57 | 1.05 | 0.07 | 0.01 | 0.55 | -0.86 |
| Aminoacyl-tRNA biosynthesis | Tryptophanyl-tRNA synthetase (EC 6.1.1.2) | 0.52 | 0.89 | -0.17 | 0.00 | 0.27 | -1.90 |
| Aminoacyl-tRNA biosynthesis | Histidyl-tRNA synthetase (EC 6.1.1.21) (Histidine--tRNA ligase) (HisRS) | 0.52 | 1.17 | 0.23 | 0.01 | 0.32 | -1.63 |

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|---|---|---|---|---|---|---|---|
| Aminoacyl-tRNA biosynthesis | CBS domain-containing protein | 0.21 | 2.33 | 1.22 | 0.03 | 2.33 | 1.22 |
| Apoptosis | Superfamily II DNA and RNA helicase | 0.00 | 4.29 | 2.10 | 0.61 | 1.18 | 0.24 |
| Arginine and proline metabolism | Dipeptidase A, Cysteine peptidase, MEROPS family C69 | 0.00 | 6.07 | 2.60 | 0.00 | 4.73 | 2.24 |
| Arginine and proline metabolism | Xaa-Pro dipeptidase (Proline dipeptidase) | 0.00 | 2.60 | 1.38 | 0.00 | 3.67 | 1.87 |
| Arginine and proline metabolism | Dipeptidase D-type (U34 family) (EC 3.4.-.-) | 0.00 | 6.34 | 2.66 | 0.00 | 4.73 | 2.24 |
| Arginine and proline metabolism | Proline iminopeptidase (PIP) (EC 3.4.11.5) (Prolyl aminopeptidase) | 0.19 | 0.84 | -0.26 | 0.01 | 0.51 | -0.97 |
| Arginine and proline metabolism | Proline iminopeptidase (PIP) (EC 3.4.11.5) (Prolyl aminopeptidase) | 0.19 | 0.84 | -0.26 | 0.01 | 0.51 | -0.97 |
| Arginine and proline metabolism | Oxaloacetate decarboxylase, alpha subunit (EC 4.1.1.3) | 0.31 | 1.57 | 0.66 | 0.00 | 2.02 | 1.02 |
| Bacterial secretion system | Signal recognition particle protein Ffh | 0.03 | 3.32 | 1.73 | 0.02 | 2.42 | 1.27 |
| Cell cycle | ATP-dependent RNA helicase | 0.00 | 4.59 | 2.20 | 0.49 | 1.24 | 0.31 |
| Cell cycle | ATP-dependent zinc metalloprotease FtsH (EC 3.4.24.-) | 0.24 | 1.13 | 0.18 | 0.04 | 0.58 | -0.79 |
| cell cycle regulation | Hit-like protein involved in cell-cycle regulation | 0.23 | 2.39 | 1.26 | 0.01 | 2.74 | 1.45 |
| Cell division protein | Cell division protein | 0.56 | 1.09 | 0.13 | 0.00 | 0.35 | -1.52 |
| Citrate cycle | Putative citrate lyase alpha subunit (EC 4.1.3.6) (EC 2.8.3.10) | 0.59 | 1.17 | 0.22 | 0.00 | 2.48 | 1.31 |
| Citrate cycle | Citrate lyase, beta subunit (EC 4.1.3.6) | 0.77 | 1.34 | 0.42 | 0.00 | 3.14 | 1.65 |
| Citrate cycle | Citrate lyase, alpha subunit | 0.59 | 1.17 | 0.22 | 0.00 | 2.47 | 1.30 |
| Cold shock protein | Cold shock protein A (Cold-shock protein) | 0.13 | 0.36 | -1.47 | 0.00 | 0.66 | -0.61 |
| Cyanoamino acid metabolism | Beta-glucoside operon | 0.01 | 0.54 | -0.89 | 0.00 | 0.04 | -4.81 |
| Cysteine and methionine metabolism | Aspartate/tyrosine/aromatic aminotransferase | 0.28 | 0.94 | -0.09 | 0.01 | 0.37 | -1.45 |
| Cysteine and methionine metabolism | Cysteine synthase (EC 2.5.1.47) | 0.00 | 0.12 | -3.01 | 0.00 | 0.08 | -3.57 |
| Cysteine and methionine metabolism | Homoserine trans-succinylase | 0.03 | 0.48 | -1.05 | 0.02 | 0.48 | -1.05 |

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|---|---|---|---|---|---|---|---|
| Cysteine and methionine metabolism | Homoserine O-succinyltransferase (EC 2.3.1.46) | 0.03 | 0.48 | -1.05 | 0.02 | 0.48 | -1.05 |
| D-Alanine metabolism | D-alanine--D-alanine ligase (EC 6.3.2.4) (D-Ala-D-Ala ligase) (D-alanylalanine synthetase) | 0.15 | 1.52 | 0.61 | 0.00 | 2.20 | 1.14 |
| D-Glutamine and D-glutamate metabolism | UDP-N-acetylmuramoylalanine--D-glutamate ligase (EC 6.3.2.9) (D-glutamic acid-adding enzyme) (UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase) | 0.36 | 1.10 | 0.13 | 0.03 | 0.52 | -0.95 |
| D-Glutamine and D-glutamate metabolism | UDP-N-acetylmuramoylalanine--D-glutamate ligase (EC 6.3.2.9) (D-glutamic acid-adding enzyme) (UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase) | 0.36 | 1.10 | 0.13 | 0.03 | 0.52 | -0.95 |
| D-Glutamine and D-glutamate metabolism | Uncharacterized protein | 0.36 | 1.10 | 0.13 | 0.03 | 0.52 | -0.95 |
| DNA replication proteins | Histone-like DNA-binding protein | 0.04 | 1.02 | 0.03 | 0.48 | 1.05 | 0.08 |
| DNA replication proteins | DNA-binding protein HU-beta-like protein | 0.04 | 1.02 | 0.03 | 0.48 | 1.05 | 0.08 |
| DNA replication proteins | Single-stranded DNA-binding | 0.03 | 0.43 | -1.22 | 0.44 | 0.77 | -0.37 |
| DNA replication proteins | DNA gyrase subunit B (EC | 0.09 | 0.70 | -0.51 | 0.01 | 0.48 | -1.07 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100) | 0.04 | 0.52 | -0.95 | 0.08 | 1.57 | 0.65 |
| Fatty acid biosynthesis | Fatty acid/phospholipid biosynthesis enzyme | 0.02 | 0.66 | -0.60 | 0.02 | 0.44 | -1.20 |
| Fatty acid biosynthesis | Acyl carrier protein 2 (ACP 2) | 0.09 | 0.93 | -0.10 | 0.03 | 1.72 | 0.78 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier-protein] synthase II (EC 2.3.1.41) | 0.07 | 1.03 | 0.04 | 0.00 | 2.33 | 1.22 |
| Fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier-protein] synthase 2 (EC 2.3.1.179) | 0.06 | 1.04 | 0.06 | 0.00 | 2.40 | 1.26 |
| Fatty acid biosynthesis | Biotin carboxylase | 0.70 | 2.37 | 1.25 | 0.00 | 4.20 | 2.07 |
| Fatty acid biosynthesis | 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.-) | 0.49 | 3.53 | 1.82 | 0.00 | 3.95 | 1.98 |
| Fatty acid biosynthesis | [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39) | 0.55 | 1.28 | 0.36 | 0.01 | 2.68 | 1.42 |

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|---|---|---|---|---|---|---|---|
| Fatty acid biosynthesis | Malonyl CoA-acyl carrier protein transacylase (EC 2.3.1.39) | 0.58 | 1.31 | 0.39 | 0.01 | 2.74 | 1.46 |
| Fatty acid metabolism | Acetyl-CoA acetyltransferase (Acetoacetyl-CoA thiolase) (EC 2.3.1.9) | 0.18 | 3.13 | 1.65 | 0.00 | 5.80 | 2.54 |
| Fatty acid metabolism | Acetyl-CoA acetyltransferase | 0.18 | 1.80 | 0.85 | 0.00 | 4.20 | 2.07 |
| Fatty acid metabolism | Acetyl-CoA carboxylase carboxyltransferase subunit alpha (EC 6.3.4.14) | 0.70 | 2.37 | 1.25 | 0.00 | 4.20 | 2.07 |
| Fatty acid metabolism | Acetyl-CoA carboxylase, biotin carboxyl carrier protein | 0.88 | 2.78 | 1.47 | 0.00 | 3.96 | 1.99 |
| Fructose and mannose metabolism | PTS system D-fructose-specific IIC component (F1P-forming), Frc family / PTS system D-fructose-specific IIB component (F1P-forming), Frc family / PTS system D-fructose-specific IIA component | 0.82 | 1.12 | 0.16 | 0.00 | 0.22 | -2.16 |
| Fructose and mannose metabolism | Mannose-6-phosphate isomerase, type 1 (EC 5.3.1.8) | 0.68 | 0.78 | -0.37 | 0.01 | 0.48 | -1.07 |
| Fructose and mannose metabolism | Mannose-6-phosphate isomerase (EC 5.3.1.8) | 0.73 | 0.79 | -0.34 | 0.01 | 0.44 | -1.17 |
| Fructose and mannose metabolism | EIIAB | 0.08 | 0.65 | -0.63 | 0.01 | 0.67 | -0.58 |
| Fructose and mannose metabolism | 1-phosphofructokinase (Fructose-1-phosphate kinase) (EC 2.7.1.56) | 0.80 | 0.87 | -0.19 | 0.00 | 0.21 | -2.23 |
| Galactose metabolism | N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25) | 0.00 | 1.70 | 0.77 | 0.15 | 1.43 | 0.51 |
| Galactose metabolism | N-acetylglucosamine-6-phosphate deacetylase | 0.02 | 3.93 | 1.98 | 0.18 | 1.53 | 0.62 |
| Galactose metabolism | Tagatose-6-phosphate kinase | 0.70 | 0.89 | -0.17 | 0.00 | 0.24 | -2.04 |
| Galactose metabolism | LacE | 0.15 | 2.03 | 1.02 | 0.02 | 0.48 | -1.05 |
| Galactose metabolism | LacE | 0.15 | 2.03 | 1.02 | 0.02 | 0.48 | -1.05 |
| Galactose metabolism | Aldo/keto reductase related | 0.02 | 0.47 | -1.09 | 0.01 | 0.60 | -0.75 |

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|---|---|---|---|---|---|---|---|
| Galactose metabolism | 6-phospho-beta-galactosidase (EC 3.2.1.85) (Beta-D-phosphogalactoside galactohydrolase) (PGALase) (P-beta-Gal) (PBG) | 0.02 | 0.48 | -1.05 | 0.00 | 0.09 | -3.44 |
| Galactose metabolism | Lactose-specific phosphotransferase enzyme IIA component (PTS system lactose-specific EIIA component) (EIIA-LacG | 0.01 | 0.86 | -0.21 | 0.00 | 0.08 | -3.59 |
| Galactose metabolism | | 0.00 | 0.40 | -1.33 | 0.00 | 0.10 | -3.29 |
| Galactose metabolism | Lactose-specific phosphotransferase enzyme IIA component (EC 2.7.1.-) (EIIA-Lac) (EIII-Lac) (PTS system lactose- | 0.01 | 0.86 | -0.21 | 0.00 | 0.08 | -3.59 |
| Galactose metabolism | Aldo/keto reductase of diketogulonate reductase family | 0.00 | 0.34 | -1.56 | 0.00 | 0.29 | -1.78 |
| Galactose metabolism | Aldo/keto reductase of diketogulonate reductase family | 0.00 | 0.36 | -1.47 | 0.00 | 0.31 | -1.69 |
| Galactose metabolism | Aldo/keto reductase (Fragment) | 0.00 | 0.35 | -1.52 | 0.00 | 0.26 | -1.94 |
| Galactose metabolism | Aldo/keto reductase (Fragment) | 0.00 | 0.32 | -1.65 | 0.00 | 0.24 | -2.07 |
| Galactose metabolism | Lactose phosphotransferase system repressor | 0.00 | 0.53 | -0.92 | 0.00 | 0.05 | -4.39 |
| Galactose metabolism | Galactokinase (EC 2.7.1.6) (Galactose kinase) | 0.15 | 0.98 | -0.02 | 0.00 | 2.21 | 1.14 |
| Galactose metabolism | Galactokinase (EC 2.7.1.6) (Galactose kinase) | 0.29 | 1.16 | 0.21 | 0.00 | 2.36 | 1.24 |
| Galactose metabolism | UDP-glucose 4-epimerase (EC 5.1.3.2) (Galactowaldenase) (UDP-galactose 4-epimerase) | 0.63 | 0.81 | -0.30 | 0.00 | 2.10 | 1.07 |
| Galactose metabolism | Galactose-1-phosphate uridylyltransferase (Gal-1-P uridylyltransferase) (EC 2.7.7.12) (UDP-glucose--hexose-1-phosphate uridylyltransferase) | 0.79 | 1.23 | 0.30 | 0.04 | 1.49 | 0.58 |

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|---|---|---|---|---|---|---|---|
| Galactose metabolism | Galactose-1-phosphate uridylyltransferase (Gal-1-P uridylyltransferase) (EC 2.7.7.12) (UDP-glucose--hexose-1-phosphate uridylyltransferase) | 0.55 | 1.07 | 0.10 | 0.02 | 1.45 | 0.54 |
| Galactose metabolism | UDP-galactose 4-epimerase (EC 5.1.3.2) | 0.88 | 0.77 | -0.39 | 0.00 | 2.39 | 1.26 |
| Galactose metabolism | Uncharacterized protein | 0.79 | 1.23 | 0.30 | 0.04 | 1.49 | 0.58 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) (D-tagatose-1,6-bisphosphate aldolase 1) (Tagatose-bisphosphate aldolase | 0.95 | 0.89 | -0.16 | 0.00 | 0.25 | -1.98 |
| Galactose metabolism | Galactose-6-phosphate isomerase lacB subunit (EC 5.3.1.26) | 0.45 | 1.24 | 0.31 | 0.00 | 0.09 | -3.50 |
| Galactose metabolism | Galactose-6-phosphate isomerase lacA subunit (EC 5.3.1.26) | 0.10 | 0.90 | -0.15 | 0.00 | 0.07 | -3.84 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) (D-tagatose-1,6-bisphosphate aldolase 1) (Tagatose-bisphosphate aldolase | 0.97 | 0.86 | -0.21 | 0.00 | 0.20 | -2.34 |
| Galactose metabolism | Galactose-6-phosphate isomerase subunit lacB (EC 5.3.1.26) | 0.43 | 1.24 | 0.31 | 0.00 | 0.08 | -3.58 |
| Galactose metabolism | Galactose-6-phosphate isomerase subunit lacA (EC 5.3.1.26) | 0.10 | 0.90 | -0.15 | 0.00 | 0.07 | -3.84 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 2 (EC 4.1.2.40) (D-tagatose-1,6-bisphosphate aldolase 2) (Tagatose-bisphosphate aldolase | 0.39 | 0.75 | -0.41 | 0.01 | 0.74 | -0.43 |
| Galactose metabolism | Galactokinase 1 (EC 2.7.1.6) (Galactose kinase 1) | 0.31 | 1.09 | 0.13 | 0.00 | 2.26 | 1.18 |
| Galactose metabolism | Galactose-1-phosphate uridylyltransferase 1 (Gal-1-P uridylyltransferase 1) (EC 2.7.7.12) (UDP-glucose--hexose-1-phosphate uridylyltransferase 1) | 0.55 | 1.07 | 0.10 | 0.02 | 1.45 | 0.54 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Galactose metabolism | Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24) | 0.17 | 1.56 | 0.64 | 0.01 | 2.01 | 1.01 |
| Galactose metabolism | Galactokinase (EC 2.7.1.6) (Galactose kinase) | 0.31 | 1.09 | 0.13 | 0.00 | 2.26 | 1.17 |
| Galactose metabolism | Galactose-1-phosphate uridylyltransferase (Gal-1-P uridylyltransferase) (EC 2.7.7.12) (UDP-glucose--hexose-1-phosphate uridylyltransferase) | 0.79 | 1.14 | 0.19 | 0.03 | 1.49 | 0.58 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) (D-tagatose-1,6-bisphosphate aldolase 1) (Tagatose-bisphosphate aldolase | 0.84 | 0.89 | -0.16 | 0.00 | 0.26 | -1.95 |
| Galactose metabolism | Galactose 6-phosphate isomerase | 0.07 | 0.91 | -0.14 | 0.00 | 0.08 | -3.73 |
| Galactose metabolism | Tagatose 1,6-diphosphate aldolase 1 (EC 4.1.2.40) (D-tagatose-1,6-bisphosphate aldolase 1) (Tagatose-bisphosphate aldolase | 0.93 | 0.87 | -0.20 | 0.00 | 0.18 | -2.44 |
| Galactose metabolism | Sugar-phosphate isomerase, RpiB/LacA/LacB family | 0.07 | 0.91 | -0.14 | 0.00 | 0.08 | -3.73 |
| Galactose metabolism | Phosphocarrier protein HPr (EC 2.7.11.-) (Histidine-containing protein) | 0.28 | 1.06 | 0.08 | 0.03 | 0.63 | -0.66 |
| Galactose metabolism | Tagatose-6-phosphate kinase (Phosphotagatokinase) (EC 2.7.1.144) | 0.65 | 0.74 | -0.44 | 0.00 | 0.09 | -3.48 |
| Galactose metabolism | Tagatose-6-phosphate kinase (EC 2.7.1.144) | 0.85 | 0.69 | -0.53 | 0.00 | 0.07 | -3.74 |
| Galactose metabolism | Tagatose-6-phosphate kinase | 0.65 | 0.71 | -0.49 | 0.00 | 0.06 | -4.01 |
| Galactose metabolism | Fructose-specific phosphotransferase system, enzyme IIABC (EC 2.7.1.69) | 0.77 | 1.10 | 0.14 | 0.00 | 0.19 | -2.40 |
| Galactose metabolism | Lactose-specific IIC component | 0.07 | 2.03 | 1.02 | 0.02 | 0.48 | -1.05 |

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|---|---|---|---|---|---|---|---|
| Galactose metabolism | PTS system lactose-specific EIICB component (EIICB-Lac) (EII-Lac) [Includes: Lactose permease IIC component (PTS system lactose-specific EIIC component); Lactose-specific phosphotransferase enzyme IIB component (EC | 0.15 | 2.03 | 1.02 | 0.02 | 0.48 | -1.05 |
| Glutathione metabolism | Glutathione reductase | 0.04 | 1.19 | 0.25 | 0.64 | 1.19 | 0.25 |
| Glutathione metabolism | Lysyl aminopeptidase, Metallo peptidase, MEROPS family M01 | 0.23 | 1.00 | 0.00 | 0.00 | 0.30 | -1.75 |
| Glutathione metabolism | Aminopeptidase N | 0.20 | 0.97 | -0.04 | 0.00 | 0.28 | -1.83 |
| Glycerolipid metabolism | Phosphate acyltransferase (EC 2.3.1.n2) (Acyl-ACP phosphotransacylase) (Acyl-[acyl-carrier-protein]--phosphate acyltransferase) (Phosphate-acyl-ACP acyltransferase) | 0.02 | 0.66 | -0.60 | 0.02 | 0.44 | -1.20 |
| Glycerolipid metabolism | Phosphate acyltransferase (EC 2.3.1.n2) (Acyl-ACP phosphotransacylase) (Acyl-[acyl-carrier-protein]--phosphate acyltransferase) (Phosphate-acyl-ACP acyltransferase) | 0.02 | 0.66 | -0.60 | 0.02 | 0.44 | -1.20 |
| Glycerolipid metabolism | Glycerol kinase 1 (EC 2.7.1.30) (ATP:glycerol 3-phosphotransferase 1) | 1.00 | 1.00 | 0.00 | 0.00 | 4.20 | 2.07 |
| Glycerolipid metabolism | Esterase/lipase | 0.00 | 0.35 | -1.52 | 0.00 | 0.35 | -1.52 |
| Glycerolipid metabolism | Esterase/lipase | 0.03 | 0.74 | -0.43 | 0.02 | 0.48 | -1.05 |
| Glycerophospholipid metabolism | Glycerol 3-phosphate oxidase (EC 1.1.3.21) | 1.00 | 1.00 | 0.00 | 0.00 | 5.53 | 2.47 |
| Glycerophospholipid metabolism | Glycerol-3-phosphate dehydrogenase | 1.00 | 1.00 | 0.00 | 0.00 | 5.53 | 2.47 |
| Glycerophospholipid metabolism | Predicted esterase | 0.03 | 0.48 | -1.05 | 0.02 | 0.48 | -1.05 |
| Glycerophospholipid metabolism | Glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) | 1.00 | 1.00 | 0.00 | 0.00 | 5.00 | 2.32 |

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| Glycolysis / Gluconeogenesis | NADPH:quinone reductase related Zn-dependent oxidoreductase | 0.00 | 0.32 | -1.65 | 0.00 | 0.32 | -1.65 |
| Glycolysis / Gluconeogenesis | Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) | 0.12 | 1.84 | 0.88 | 0.00 | 3.32 | 1.73 |
| Glycolysis / Gluconeogenesis | Fructose-bisphosphate aldolase (EC 4.1.2.13) | 0.00 | 1.60 | 0.68 | 0.01 | 1.37 | 0.45 |
| Glycolysis / Gluconeogenesis | Glucokinase (EC 2.7.1.2) | 0.02 | 1.61 | 0.69 | 0.00 | 2.52 | 1.33 |
| Glycolysis / Gluconeogenesis | Galactose mutarotase related enzyme | 0.04 | 0.65 | -0.63 | 0.01 | 0.61 | -0.70 |
| Glycolysis / Gluconeogenesis | Fructose-bisphosphate aldolase (EC 4.1.2.13) | 0.00 | 1.57 | 0.65 | 0.03 | 1.32 | 0.40 |
| Glycolysis / Gluconeogenesis | 2,5-diketo-D-gluconate reductase (EC 1.1.1.274) | 0.00 | 0.45 | -1.14 | 0.00 | 0.56 | -0.83 |
| Glycolysis / Gluconeogenesis | Glyceraldehyde 3-phosphate dehydrogenase | 0.03 | 1.81 | 0.85 | 0.00 | 2.15 | 1.11 |
| Glycolysis / Gluconeogenesis | Phosphoglycerate kinase (EC 2.7.2.3) | 0.01 | 1.43 | 0.52 | 0.03 | 1.35 | 0.43 |
| Glycolysis / Gluconeogenesis | Triosephosphate isomerase (TIM) (EC 5.3.1.1) (Triose-phosphate isomerase) | 0.00 | 1.06 | 0.09 | 0.00 | 1.54 | 0.63 |
| Glycolysis / Gluconeogenesis | Glucokinase (EC 2.7.1.2) | 0.02 | 1.61 | 0.69 | 0.00 | 2.52 | 1.33 |
| Glycolysis / Gluconeogenesis | Galactose mutarotase related enzyme | 0.03 | 0.64 | -0.64 | 0.02 | 0.62 | -0.68 |
| Glycolysis / Gluconeogenesis | Phosphoglycerate kinase (EC 2.7.2.3) | 0.01 | 1.43 | 0.52 | 0.03 | 1.35 | 0.43 |
| Glycolysis / Gluconeogenesis | Triosephosphate isomerase (TIM) (EC 5.3.1.1) (Triose-phosphate isomerase) | 0.00 | 1.06 | 0.09 | 0.00 | 1.54 | 0.63 |
| Glycolysis / Gluconeogenesis | Oxidoreductase | 0.00 | 0.34 | -1.56 | 0.00 | 0.29 | -1.78 |
| Glycolysis / Gluconeogenesis | 6-phospho-beta-glucosidase | 0.03 | 4.47 | 2.16 | 0.03 | 2.33 | 1.22 |
| Glycolysis / Gluconeogenesis | 6-phospho-beta-glucosidase (EC 3.2.1.86) | 0.00 | 3.40 | 1.77 | 0.03 | 2.07 | 1.05 |
| Glycolysis / Gluconeogenesis | Glucose-6-phosphate isomerase (GPI) (EC 5.3.1.9) (Phosphoglucose isomerase) (PGI) (Phosphohexose isomerase) (PHI) | 0.02 | 0.69 | -0.54 | 0.11 | 1.20 | 0.26 |

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| Glycolysis / Gluconeogenesis | Pyruvate dehydrogenase complex, E1 component, alpha subunit (EC 1.2.4.1) | 0.05 | 0.62 | -0.68 | 0.97 | 1.01 | 0.01 |
| Glycolysis / Gluconeogenesis | Galactose mutarotase related enzyme | 0.04 | 0.86 | -0.22 | 0.14 | 0.69 | -0.54 |
| Glycolysis / Gluconeogenesis | Oxidoreductase | 0.03 | 0.61 | -0.72 | 0.27 | 0.85 | -0.24 |
| Glycolysis / Gluconeogenesis | Glucose-6-phosphate isomerase (EC 5.3.1.9) | 0.02 | 0.69 | -0.54 | 0.11 | 1.20 | 0.26 |
| Glycolysis / Gluconeogenesis | Glucose-6-phosphate isomerase (EC 5.3.1.9) | 0.02 | 0.69 | -0.54 | 0.11 | 1.20 | 0.26 |
| Glycolysis / Gluconeogenesis | Glucose-6-phosphate isomerase (EC 5.3.1.9) (Fragment) | 0.01 | 0.67 | -0.57 | 0.16 | 1.19 | 0.25 |
| Glycolysis / Gluconeogenesis | Galactose mutarotase related enzyme | 0.01 | 0.84 | -0.24 | 0.12 | 0.65 | -0.62 |
| Glycolysis / Gluconeogenesis | Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (2-phosphoglycerate dehydratase) | 0.62 | 1.13 | 0.18 | 0.01 | 1.44 | 0.53 |
| Glycolysis / Gluconeogenesis | Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (2-phosphoglycerate dehydratase) | 0.59 | 1.15 | 0.20 | 0.01 | 1.44 | 0.53 |
| Glycolysis / Gluconeogenesis | GalM | 0.52 | 0.64 | -0.64 | 0.00 | 1.80 | 0.85 |
| Glycolysis / Gluconeogenesis | Aldose 1-epimerase | 0.80 | 0.71 | -0.50 | 0.00 | 1.82 | 0.86 |
| Glycolysis / Gluconeogenesis | Uncharacterized protein | 0.13 | 0.62 | -0.69 | 0.01 | 0.58 | -0.79 |
| Glycolysis / Gluconeogenesis | Mutarotase (Fragment) | 0.61 | 0.71 | -0.49 | 0.00 | 1.81 | 0.86 |
| Glycolysis / Gluconeogenesis | Predicted oxidoreductase | 0.43 | 0.69 | -0.53 | 0.01 | 0.38 | -1.38 |
| Glycolysis / Gluconeogenesis | Phosphotransferase system, mannose/fructose/N-acetylgalactosamine-specific component IIB | 0.11 | 0.57 | -0.81 | 0.00 | 0.09 | -3.48 |
| Glycolysis / Gluconeogenesis | Fusion of IIA, IIB and IIC component of mannitol/fructose-specific phosphotransferase system mannitol/fructose-specific | 0.77 | 1.10 | 0.14 | 0.00 | 0.19 | -2.40 |
| Glycolysis / Gluconeogenesis | PTS system, lactose-specific IIBC component | 0.15 | 2.03 | 1.02 | 0.02 | 0.48 | -1.05 |

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| Glycolysis / Gluconeogenesis | PTS system, lactose-specific IIBC component | 0.10 | 1.91 | 0.94 | 0.01 | 0.43 | -1.22 |
| Glycolysis / Gluconeogenesis | Fructose-1,6-bisphosphatase class 3 (FBPase class 3) (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase class 3) | 0.18 | 2.33 | 1.22 | 0.00 | 4.73 | 2.24 |
| Glycolysis / Gluconeogenesis | Fructose-1,6-bisphosphatase class 3 (FBPase class 3) (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase class 3) | 0.18 | 2.07 | 1.05 | 0.00 | 4.20 | 2.07 |
| Glycolysis / Gluconeogenesis | Alcohol-acetaldehyde dehydrogenase | 1.00 | 2.33 | 1.22 | 0.02 | 2.07 | 1.05 |
| Glycolysis / Gluconeogenesis | L-lactate dehydrogenase 2 (L-LDH 2) (EC 1.1.1.27) | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Glycolysis / Gluconeogenesis | Phosphoenolpyruvate carboxykinase (ATP) | 1.00 | 2.07 | 1.05 | 0.02 | 2.33 | 1.22 |
| Glycosyltransferases | 3-oxoacyl-[acyl-carrier-protein] synthase 3 (EC 2.3.1.180) (3-oxoacyl-[acyl-carrier-protein] synthase III) (Beta-ketoacyl-ACP | 0.16 | 3.13 | 1.65 | 0.00 | 4.47 | 2.16 |
| GTP-binding proteins | GTPase obg (GTP-binding protein obg) | 0.03 | 1.26 | 0.33 | 0.03 | 0.48 | -1.05 |
| GTP-binding proteins | GTP-binding protein-BipA-EF-TU family | 0.09 | 0.52 | -0.94 | 0.03 | 0.67 | -0.58 |
| GTP-binding proteins | GTPase Der (GTP-binding protein EngA) | 0.37 | 0.54 | -0.88 | 0.03 | 0.43 | -1.22 |
| GTP-binding proteins | GTPase Der (GTP-binding protein EngA) | 0.37 | 0.54 | -0.88 | 0.03 | 0.43 | -1.22 |
| Heat shock proteins | Hsp60 (Fragment) | 0.08 | 0.60 | -0.73 | 0.03 | 0.76 | -0.39 |
| Heat shock proteins | Hsp60 (Fragment) | 0.08 | 0.60 | -0.73 | 0.03 | 0.76 | -0.39 |
| Heat shock proteins | 60 kDa heat shock protein (Fragment) | 0.07 | 0.58 | -0.78 | 0.04 | 0.76 | -0.39 |
| Heat shock proteins | 60 kDa heat shock protein (HSP60) (Fragment) | 0.08 | 0.60 | -0.73 | 0.03 | 0.76 | -0.39 |

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| Inositol phosphate metabolism | 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase (THcHDO hydrolase) (EC 3.7.1.n2) | 1.00 | 1.00 | 0.00 | 0.00 | 4.20 | 2.07 |
| Inositol phosphate metabolism | Inosose dehydratase (EC 4.2.1.44) (2-keto-myo-inositol dehydratase) (2KMI dehydratase) | 1.00 | 1.00 | 0.00 | 0.00 | 6.60 | 2.72 |
| Inositol phosphate metabolism | 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase 2 (THcHDO hydrolase 2) (EC 3.7.1.n2) | 1.00 | 1.00 | 0.00 | 0.00 | 4.20 | 2.07 |
| Inositol phosphate metabolism | 5-dehydro-2-deoxygluconokinase 2 (EC 2.7.1.92) (2-deoxy-5-keto-D-gluconate kinase 2) | 1.00 | 1.00 | 0.00 | 0.00 | 3.40 | 1.77 |
| Inositol phosphate metabolism | 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase 3 (THcHDO hydrolase 3) (EC 3.7.1.n2) | 1.00 | 1.00 | 0.00 | 0.00 | 3.93 | 1.98 |
| Inositol phosphate metabolism | Inosose dehydratase 3 (EC 4.2.1.44) (2-keto-myo-inositol | 1.00 | 1.00 | 0.00 | 0.00 | 5.27 | 2.40 |
| Inositol phosphate metabolism | 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase 4 (THcHDO hydrolase 4) (EC 3.7.1.n2) | 1.00 | 1.00 | 0.00 | 0.00 | 3.93 | 1.98 |
| Inositol phosphate metabolism | 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase 5 (THcHDO hydrolase 5) (EC 3.7.1.n2) | 1.00 | 1.00 | 0.00 | 0.00 | 4.20 | 2.07 |
| Inositol phosphate metabolism | Inosose dehydratase 4 (EC 4.2.1.44) (2-keto-myo-inositol | 1.00 | 1.00 | 0.00 | 0.00 | 6.60 | 2.72 |
| Lipid Metabolism | Predicted flavoprotein | 0.03 | 0.48 | -1.05 | 0.40 | 0.74 | -0.43 |
| Lipid Metabolism | Predicted flavoprotein | 0.03 | 0.43 | -1.22 | 0.30 | 0.66 | -0.61 |
| Lysine biosynthesis | UDP-N-acetylmuramyl-tripeptide synthetase (EC 6.3.2.-) (UDP-MurNAc-tripeptide synthetase) | 0.06 | 2.05 | 1.04 | 0.03 | 2.26 | 1.18 |
| Lysine biosynthesis | Aminotransferase (EC 2.6.1.-) | 0.28 | 0.94 | -0.09 | 0.01 | 0.37 | -1.45 |
| Lysine degradation | Predicted dehydrogenase related protein | 0.35 | 0.89 | -0.18 | 0.02 | 0.43 | -1.22 |
| Membrane protease | Membrane protease subunit, stomatin/prohibitin family | 0.77 | 1.51 | 0.60 | 0.00 | 0.38 | -1.38 |

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| Metabolism of Other Amino Acids | Dihydroorotate oxidase B, catalytic subunit (EC 1.3.3.1) | 0.14 | 0.61 | -0.72 | 0.00 | 0.29 | -1.77 |
| Nicotinate and nicotinamide metabolism | Amidophosphoribosyltransferase (ATase) (EC 2.4.2.14) (Glutamine phosphoribosylpyrophosphate amidotransferase) | 0.01 | 0.38 | -1.38 | 0.00 | 0.38 | -1.38 |
| Nicotinate and nicotinamide metabolism | Nicotinate phosphoribosyltransferase (EC | 0.02 | 0.64 | -0.64 | 0.26 | 0.70 | -0.51 |
| Nicotinate and nicotinamide metabolism | Nicotinate phosphoribosyltransferase (EC | 0.03 | 0.71 | -0.50 | 0.49 | 0.78 | -0.36 |
| Nicotinate and nicotinamide metabolism | Purine nucleoside phosphorylase deoD-type (PNP) (EC 2.4.2.1) | 0.30 | 1.87 | 0.90 | 0.02 | 2.39 | 1.26 |
| Nicotinate and nicotinamide metabolism | Orotate phosphoribosyltransferase (OPRT) (OPRTase) (EC 2.4.2.10) | 0.06 | 0.56 | -0.85 | 0.04 | 0.56 | -0.85 |
| Oxidative phosphorylation | ATP synthase subunit alpha (EC 3.6.3.14) (ATP synthase F1 sector subunit alpha) (F-ATPase subunit alpha) | 0.22 | 0.68 | -0.56 | 0.02 | 0.59 | -0.75 |
| Oxidative phosphorylation | CTP synthase (UTP--ammonia ligase) (CTP synthetase) (EC 6.3.4.2) | 0.04 | 1.89 | 0.92 | 0.03 | 1.83 | 0.87 |
| Oxidative phosphorylation | NADH oxidase (EC 1.6.99.3) | 0.00 | 7.14 | 2.84 | 0.00 | 11.14 | 3.48 |
| Oxidative phosphorylation | Putative NADH-flavin reductase | 0.00 | 0.39 | -1.35 | 0.00 | 0.29 | -1.78 |
| Oxidative phosphorylation | ATP synthase subunit alpha (EC 3.6.3.14) (ATP synthase F1 sector subunit alpha) (F-ATPase subunit alpha) | 0.22 | 0.68 | -0.56 | 0.02 | 0.59 | -0.75 |
| Oxidative phosphorylation | ATP synthase subunit delta (ATP synthase F(1) sector subunit delta) (F-type ATPase subunit delta) (F-ATPase subunit delta) | 0.22 | 0.59 | -0.76 | 0.00 | 0.38 | -1.38 |
| Oxidative phosphorylation | ATP synthase subunit delta (ATP synthase F(1) sector subunit delta) (F-type ATPase subunit delta) (F-ATPase subunit delta) | 0.22 | 0.59 | -0.76 | 0.00 | 0.38 | -1.38 |

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| Pentose and glucuronate interconversions | UTP--glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) | 0.01 | 0.39 | -1.37 | 0.00 | 0.46 | -1.12 |
| Pentose and glucuronate interconversions | UDP-glucose pyrophosphorylase (EC 2.7.7.9) | 0.01 | 0.38 | -1.39 | 0.01 | 0.48 | -1.05 |
| Pentose phosphate pathway | 6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44) | 0.31 | 1.05 | 0.07 | 0.04 | 1.40 | 0.48 |
| Pentose phosphate pathway | 6-phosphogluconate dehydrogenase, decarboxylating (EC 1.1.1.44) | 0.08 | 1.16 | 0.21 | 0.05 | 1.42 | 0.51 |
| Pentose phosphate pathway | Predicted phosphatase | 1.00 | 1.00 | 0.00 | 0.04 | 1.80 | 0.85 |
| Peptidoglycan biosynthesis | Serine-type D-Ala-D-Ala carboxypeptidase (EC 3.4.16.4) | 0.00 | 5.26 | 2.40 | 0.06 | 2.16 | 1.11 |
| Peptidoglycan biosynthesis | FemAB family protein | 0.02 | 2.33 | 1.22 | 1.00 | 1.00 | 0.00 |
| Phosphotransferase system | Cellobiose-specific PTS system IIC component | 0.04 | 1.91 | 0.94 | 0.01 | 0.43 | -1.22 |
| Phosphotransferase system | Beta-glucoside bgl operon antiterminator, BglG family (Transcription antiterminator LacT) | 0.01 | 0.53 | -0.91 | 0.00 | 0.04 | -4.75 |
| Phosphotransferase system | Transcription antiterminator LacT | 0.01 | 0.54 | -0.89 | 0.00 | 0.04 | -4.81 |
| Phosphotransferase system | Phosphotransferase system sugar-specific EII component | 0.02 | 0.75 | -0.42 | 0.00 | 0.16 | -2.66 |
| Phosphotransferase system | Phosphotransferase system galacitol-specific IIA domain (Ntr- | 0.02 | 0.71 | -0.50 | 0.00 | 0.16 | -2.66 |
| Phosphotransferase system | Fructose/mannose phosphotransferase system IIB component | 0.09 | 0.57 | -0.81 | 0.00 | 0.08 | -3.70 |
| Phosphotransferase system | Phosphotransferase system, galactitol-specific IIB component | 0.09 | 0.63 | -0.67 | 0.00 | 0.08 | -3.70 |
| Predicted membrane protein | Predicted membrane protein | 0.33 | 1.39 | 0.47 | 0.02 | 0.48 | -1.05 |
| Propanoate metabolism | Pyruvate/oxaloacetate carboxyltransferase | 0.25 | 1.70 | 0.76 | 0.00 | 2.20 | 1.14 |
| Propanoate metabolism | Pyruvate/oxaloacetate carboxyltransferase | 0.25 | 1.70 | 0.76 | 0.00 | 2.20 | 1.14 |
| protein degradation | Peptidase M3B, oligoendopeptidase F | 0.00 | 0.52 | -0.94 | 0.06 | 0.65 | -0.63 |

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| protein degradation | Oligoendopeptidase F | 0.00 | 0.52 | -0.94 | 0.06 | 0.65 | -0.63 |
| protein degradation | Oligoendopeptidase F1 (EC 3.4.24.- | 0.13 | 0.70 | -0.52 | 0.01 | 0.44 | -1.17 |
| protein degradation | Peptidase, S9 family | 0.00 | 5.80 | 2.54 | 0.00 | 6.07 | 2.60 |
| protein degradation | Dipeptidyl aminopeptidase/acylaminoacyl-peptidase | 0.03 | 3.40 | 1.77 | 0.00 | 3.93 | 1.98 |
| protein degradation | Dipeptidyl aminopeptidase/acylaminoacyl-peptidase | 0.03 | 3.40 | 1.77 | 0.00 | 4.73 | 2.24 |
| protein degradation | Peptidase T | 0.03 | 0.43 | -1.22 | 0.02 | 0.43 | -1.22 |
| protein degradation | Peptidase T | 0.03 | 0.43 | -1.22 | 0.02 | 0.43 | -1.22 |
| protein degradation | D-Ala-D-Ala carboxypeptidase A, Serine peptidase, MEROPS family S11 | 0.00 | 5.13 | 2.36 | 0.07 | 2.03 | 1.02 |
| protein degradation | Membrane alanine aminopeptidase (EC 3.4.11.2) | 0.23 | 1.00 | 0.00 | 0.00 | 0.31 | -1.67 |
| Protein digestion and absorption | Xaa-Pro aminopeptidase, Metallo peptidase, MEROPS family M24B | 0.00 | 3.13 | 1.65 | 0.00 | 4.73 | 2.24 |
| Protein export | Signal recognition particle subunit FFH/SRP54 (Srp54) | 0.03 | 3.32 | 1.73 | 0.02 | 2.42 | 1.27 |
| Protein export | Protein translocase subunit SecA | 0.04 | 2.45 | 1.29 | 0.44 | 0.81 | -0.31 |
| Protein export | Protein translocase subunit SecA | 0.04 | 2.45 | 1.29 | 0.44 | 0.81 | -0.31 |
| protein synthesis | Oligopeptidase F, Metallo peptidase, MEROPS family M03B | 0.00 | 0.52 | -0.94 | 0.06 | 0.65 | -0.63 |
| protein synthesis | Elongation factor G (EF-G) | 0.01 | 1.16 | 0.22 | 0.30 | 0.92 | -0.11 |
| protein synthesis | Elongation factor EF-2 (Fragment) | 0.03 | 1.19 | 0.25 | 0.75 | 0.97 | -0.04 |
| protein synthesis | Elongation factor G (EF-G) | 0.01 | 1.19 | 0.25 | 0.16 | 0.90 | -0.16 |
| protein synthesis | Elongation factor G (EF-G) | 0.01 | 1.18 | 0.23 | 0.28 | 0.92 | -0.12 |
| protein synthesis | Uncharacterized protein | 0.83 | 1.02 | 0.02 | 0.04 | 1.26 | 0.34 |
| protein synthesis | Elongation factor Tu (EF-Tu) | 0.83 | 1.02 | 0.02 | 0.04 | 1.26 | 0.34 |
| protein synthesis | Predicted GTPase, probable translation factor | 0.77 | 0.87 | -0.21 | 0.03 | 0.62 | -0.69 |
| protein synthesis | Putative elongation factor Tu (Fragment) | 0.86 | 1.13 | 0.17 | 0.05 | 1.44 | 0.53 |
| protein synthesis | Putative elongation factor Tu (Fragment) | 0.86 | 1.13 | 0.17 | 0.05 | 1.44 | 0.53 |

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| Purine metabolism | GMP synthase [glutamine-hydrolyzing] (EC 6.3.5.2) (GMP synthetase) (Glutamine amidotransferase) | 0.05 | 1.61 | 0.68 | 0.05 | 1.61 | 0.68 |
| Purine metabolism | Inosine-5'-monophosphate dehydrogenase (EC 1.1.1.205) | 0.00 | 0.36 | -1.49 | 0.00 | 0.36 | -1.49 |
| Purine metabolism | Ribonucleoside-triphosphate reductase (EC 1.17.4.2) | 0.00 | 0.16 | -2.60 | 0.00 | 0.16 | -2.60 |
| Purine metabolism | DNA-directed RNA polymerase subunit beta (RNAP subunit beta) (EC 2.7.7.6) (RNA polymerase subunit beta) (Transcriptase subunit beta) | 0.00 | 1.50 | 0.58 | 0.86 | 0.99 | -0.02 |
| Purine metabolism | DNA-directed RNA polymerase subunit beta (RNAP subunit beta) (EC 2.7.7.6) (RNA polymerase subunit beta) (Transcriptase subunit beta) | 0.00 | 1.50 | 0.58 | 0.86 | 0.99 | -0.02 |
| Purine metabolism | Adenylosuccinate synthetase (AMPSase) (AdSS) (EC 6.3.4.4) (IMP--aspartate ligase) | 0.05 | 0.54 | -0.88 | 0.00 | 0.38 | -1.39 |
| Purine metabolism | Ribonucleoside-diphosphate reductase (EC 1.17.4.1) | 0.00 | 0.35 | -1.52 | 0.00 | 0.15 | -2.70 |
| Purine metabolism | Phosphoribosylamine--glycine ligase (EC 6.3.4.13) | 0.00 | 0.13 | -2.94 | 0.00 | 0.13 | -2.94 |
| Purine metabolism | Bifunctional purine biosynthesis protein PurH [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase (EC 2.1.2.3) (AICAR transformylase); IMP cyclohydrolase (EC 3.5.4.10) (ATIC) (IMP synthase) | 0.00 | 0.11 | -3.21 | 0.00 | 0.11 | -3.21 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6) (SAICAR synthetase) | 0.00 | 0.17 | -2.54 | 0.00 | 0.17 | -2.54 |

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|---|---|---|---|---|---|---|---|
| Purine metabolism | Ribonucleoside-diphosphate reductase, beta chain (EC | 0.00 | 0.15 | -2.72 | 0.00 | 0.15 | -2.72 |
| Purine metabolism | Phosphoribosylamine--glycine ligase (GARS) (Glycinamide ribonucleotide synthetase) (Phosphoribosylglycinamide synthetase) (EC 6.3.4.13) | 0.00 | 0.10 | -3.25 | 0.00 | 0.10 | -3.25 |
| Purine metabolism | Bifunctional purine biosynthesis protein PurH [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase (EC 2.1.2.3) (AICAR transformylase); IMP cyclohydrolase (EC 3.5.4.10) (ATIC) (IMP synthase) | 0.00 | 0.09 | -3.41 | 0.00 | 0.09 | -3.41 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6) (SAICAR synthetase) | 0.00 | 0.14 | -2.89 | 0.00 | 0.14 | -2.89 |
| Purine metabolism | Ribonucleotide reductase, beta subunit | 0.00 | 0.16 | -2.66 | 0.00 | 0.16 | -2.66 |
| Purine metabolism | Phosphoribosylamine-glycine | 0.00 | 0.15 | -2.72 | 0.00 | 0.15 | -2.72 |
| Purine metabolism | Bifunctional purine biosynthesis protein PurH | 0.00 | 0.10 | -3.37 | 0.00 | 0.10 | -3.37 |
| Purine metabolism | Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6) (SAICAR synthetase) | 0.00 | 0.14 | -2.89 | 0.00 | 0.14 | -2.89 |
| Purine metabolism | Phosphoribosylformylglycinamidine (Fgam) synthase, purs component | 0.03 | 0.48 | -1.05 | 0.02 | 0.48 | -1.05 |
| Purine metabolism | Ribonucleotide reductase, alpha subunit | 0.00 | 0.16 | -2.60 | 0.00 | 0.16 | -2.60 |
| Purine metabolism | Adenosylcobalamin-dependent ribonucleoside-triphosphate reductase (RTPR) (EC 1.17.4.2) | 0.00 | 0.16 | -2.60 | 0.00 | 0.16 | -2.60 |
| Purine metabolism | Diadenosine tetraphosphate (Ap4A) hydrolase related HIT family hydrolase | 0.23 | 2.39 | 1.26 | 0.01 | 2.74 | 1.45 |

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|---|---|---|---|---|---|---|---|
| Purine metabolism | Phospho ribosylaminoimidazole carboxylase (AIR carboxylase), ATPase subunit (EC 4.1.1.21) | 0.06 | 0.85 | -0.23 | 0.04 | 0.56 | -0.85 |
| Purine metabolism | 5-(Carboxyamino)imidazole ribonucleotide synthase | 0.06 | 0.85 | -0.23 | 0.04 | 0.56 | -0.85 |
| Pyrimidine metabolism | Uncharacterized protein | 1.00 | 1.53 | 0.62 | 0.05 | 1.80 | 0.85 |
| Pyrimidine metabolism | Thymidylate synthase (TS) (TSase) (EC 2.1.1.45) | 1.00 | 1.53 | 0.62 | 0.05 | 1.80 | 0.85 |
| Pyrimidine metabolism | Thymidylate synthase (TS) (TSase) (EC 2.1.1.45) | 1.00 | 1.53 | 0.62 | 0.05 | 1.80 | 0.85 |
| Pyrimidine metabolism | Thymidylate synthase (TS) (TSase) (EC 2.1.1.45) | 1.00 | 1.53 | 0.62 | 0.05 | 1.80 | 0.85 |
| Pyrimidine metabolism | Bifunctional protein pyrR | 0.02 | 0.30 | -1.75 | 0.00 | 0.42 | -1.25 |
| Pyrimidine metabolism | Thioredoxin | 0.05 | 0.47 | -1.09 | 0.03 | 0.58 | -0.79 |
| Pyrimidine metabolism | Carbamoyl-phosphate synthase large chain (EC 6.3.5.5) (Carbamoyl-phosphate synthetase ammonia chain) | 0.01 | 0.72 | -0.48 | 0.02 | 0.44 | -1.20 |
| Pyrimidine metabolism | Carbamoyl-phosphate synthase large chain (EC 6.3.5.5) (Carbamoyl-phosphate synthetase ammonia chain) | 0.01 | 0.75 | -0.42 | 0.04 | 0.49 | -1.02 |
| Pyrimidine metabolism | Phosphomevalonate kinase (EC 2.7.4.2) | 0.00 | 2.87 | 1.52 | 0.18 | 1.53 | 0.62 |
| Pyrimidine metabolism | Phosphomevalonate kinase (EC 2.7.4.2) | 0.00 | 2.87 | 1.52 | 0.18 | 1.53 | 0.62 |
| Pyrimidine metabolism | 5'-methylthioadenosine/S-adenosylhomocysteine | 1.00 | 1.80 | 0.85 | 0.02 | 2.60 | 1.38 |
| Pyruvate metabolism | Bifunctional enzyme: alcohol dehydrogenase, acetaldehyde dehydrogenase (EC 1.1.1.1) (EC 1.2.1.10) | 0.00 | 9.98 | 3.32 | 0.00 | 4.10 | 2.04 |
| Pyruvate metabolism | Predicted acetyltransferase | 0.02 | 3.67 | 1.87 | 0.00 | 3.93 | 1.98 |
| Pyruvate metabolism | Pyruvate phosphate dikinase (EC 2.7.9.1) | 0.06 | 1.80 | 0.85 | 0.00 | 5.33 | 2.41 |

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| Pyruvate metabolism | Phosphoenolpyruvate synthase/pyruvate phosphate dikinase | 0.10 | 1.68 | 0.74 | 0.00 | 5.15 | 2.36 |
| Pyruvate metabolism | Formate C-acetyltransferase (Pyruvate formate-lyase) (Formate acetyltransferase) (EC 2.3.1.54) | 0.01 | 1.43 | 0.52 | 0.00 | 1.68 | 0.75 |
| Pyruvate metabolism | Pyruvate oxidase (EC 1.2.3.3) | 0.00 | 0.40 | -1.34 | 0.00 | 0.42 | -1.25 |
| Pyruvate metabolism | Uncharacterized NAD(FAD)-dependent dehydrogenase | 0.00 | 6.60 | 2.72 | 0.00 | 10.34 | 3.37 |
| Pyruvate metabolism | Uncharacterized NAD(FAD)-dependent dehydrogenase | 0.00 | 4.47 | 2.16 | 0.00 | 6.07 | 2.60 |
| Pyruvate metabolism | Lactoylglutathione lyase related lyase | 0.00 | 0.24 | -2.06 | 0.03 | 0.49 | -1.02 |
| Pyruvate metabolism | Acetyltransferase (EC 2.3.1.-) | 0.02 | 3.67 | 1.87 | 0.00 | 3.93 | 1.98 |
| Pyruvate metabolism | Pyruvate-formate lyase-activating enzyme | 0.02 | 3.67 | 1.87 | 0.00 | 4.73 | 2.24 |
| Pyruvate metabolism | Acetyltransferase (EC 2.3.1.-) | 0.00 | 0.49 | -1.03 | 0.00 | 0.32 | -1.65 |
| Pyruvate metabolism | Pyruvate, phosphate dikinase | 0.09 | 1.82 | 0.86 | 0.00 | 5.54 | 2.47 |
| Pyruvate metabolism | D-lactate dehydrogenase (EC 1.1.1.28) | 0.07 | 0.46 | -1.11 | 0.00 | 0.22 | -2.16 |
| Pyruvate metabolism | Pyruvate carboxylase (EC 6.4.1.1) | 1.00 | 2.60 | 1.38 | 0.05 | 2.07 | 1.05 |
| Regulatory protein | Regulatory protein, ArsR | 0.01 | 0.43 | -1.22 | 0.42 | 1.34 | 0.43 |
| Riboflavin metabolism | Acyl carrier protein 1 (ACP 1) | 0.69 | 0.87 | -0.20 | 0.05 | 0.48 | -1.05 |
| Ribosome | 50S ribosomal protein L2 | 0.00 | 1.69 | 0.76 | 0.02 | 1.61 | 0.69 |
| Ribosome | 30S ribosomal protein S3 | 0.00 | 4.46 | 2.16 | 0.01 | 2.29 | 1.19 |
| Ribosome | 50S ribosomal protein L9 | 0.00 | 5.27 | 2.40 | 0.00 | 3.13 | 1.65 |
| Ribosome | 30S ribosomal protein S14 type Z | 0.00 | 3.67 | 1.87 | 0.00 | 3.67 | 1.87 |
| Ribosome | 30S ribosomal protein S9 | 0.00 | 3.09 | 1.63 | 0.01 | 2.91 | 1.54 |
| Ribosome | 50S ribosomal protein L20 | 0.00 | 5.80 | 2.54 | 0.00 | 4.47 | 2.16 |
| Ribosome | 50S ribosomal protein L20 | 0.00 | 5.80 | 2.54 | 0.00 | 4.47 | 2.16 |
| Ribosome | 50S ribosomal protein L27 | 0.02 | 3.13 | 1.65 | 0.04 | 2.60 | 1.38 |
| Ribosome | 50S ribosomal protein L17 | 0.00 | 7.67 | 2.94 | 0.00 | 4.73 | 2.24 |
| Ribosome | SSU ribosomal protein S1P | 0.01 | 1.31 | 0.39 | 0.74 | 1.05 | 0.07 |
| Ribosome | 50S ribosomal protein L2 | 0.05 | 1.59 | 0.67 | 0.42 | 1.27 | 0.34 |
| Ribosome | 50S ribosomal protein L2 | 0.02 | 1.68 | 0.74 | 0.23 | 1.39 | 0.47 |
| Ribosome | 30S Ribosomal protein S1 | 0.01 | 1.27 | 0.35 | 0.85 | 1.04 | 0.05 |

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| Ribosome | 30S ribosomal protein S16 | 0.03 | 1.32 | 0.41 | 0.07 | 1.54 | 0.62 |
| Ribosome | 30S ribosomal protein S13 | 0.00 | 2.01 | 1.01 | 0.11 | 1.56 | 0.64 |
| Ribosome | 50S ribosomal protein L16 | 0.02 | 2.07 | 1.05 | 0.21 | 1.43 | 0.51 |
| Ribosome | 50S ribosomal protein L22 | 0.02 | 2.08 | 1.06 | 0.18 | 1.35 | 0.44 |
| Ribosome | 30S ribosomal protein S19 | 0.00 | 3.91 | 1.97 | 0.06 | 2.02 | 1.01 |
| Ribosome | 30S ribosomal protein S7 | 0.01 | 1.50 | 0.59 | 0.09 | 1.41 | 0.49 |
| Ribosome | 30S ribosomal protein S7 | 0.01 | 1.50 | 0.59 | 0.09 | 1.41 | 0.49 |
| Ribosome | 50S ribosomal protein L16 | 0.02 | 2.07 | 1.05 | 0.21 | 1.43 | 0.51 |
| Ribosome | 50S ribosomal protein L22 | 0.02 | 2.08 | 1.06 | 0.18 | 1.35 | 0.44 |
| Ribosome | 30S ribosomal protein S13 | 0.00 | 2.01 | 1.01 | 0.11 | 1.56 | 0.64 |
| Ribosome | 30S ribosomal protein S16 | 0.03 | 1.32 | 0.41 | 0.07 | 1.54 | 0.62 |
| Ribosome | 30S ribosomal protein S19 | 0.00 | 3.91 | 1.97 | 0.06 | 2.02 | 1.01 |
| Ribosome | 50S ribosomal protein L21 | 0.17 | 1.00 | 0.00 | 0.03 | 2.07 | 1.05 |
| Ribosome biogenesis in eukaryotes | Pseudouridine synthase (EC 5.4.99.-) | 0.02 | 2.60 | 1.38 | 0.18 | 1.53 | 0.62 |
| RNA degradation | mRNA degradation ribonuclease J1/J2, metallo-beta-lactamase superfamily enzyme | 0.02 | 4.47 | 2.16 | 0.00 | 2.87 | 1.52 |
| RNA degradation | 60 kDa chaperonin (GroEL protein) (Protein Cpn60) | 0.23 | 0.65 | -0.62 | 0.00 | 0.72 | -0.48 |
| RNA degradation | GroEL (Fragment) | 0.08 | 0.60 | -0.74 | 0.03 | 0.76 | -0.40 |
| RNA degradation | GroEL (Fragment) | 0.08 | 0.60 | -0.73 | 0.03 | 0.76 | -0.39 |
| RNA degradation | GroEL (Fragment) | 0.08 | 0.60 | -0.74 | 0.03 | 0.76 | -0.40 |
| RNA degradation | Predicted hydrolase of the metallo-beta-lactamase superfamily | 0.07 | 4.58 | 2.20 | 0.01 | 2.90 | 1.53 |
| RNA degradation | Ribonuclease R | 0.75 | 1.57 | 0.65 | 0.00 | 2.06 | 1.04 |
| Signal Transduction | Protein tyrosine phosphatase | 0.00 | 3.93 | 1.98 | 0.03 | 2.60 | 1.38 |
| Signal Transduction | Protein-tyrosine phosphatase (Putative) (EC 3.1.3.48) | 0.00 | 3.93 | 1.98 | 0.03 | 2.60 | 1.38 |
| Spliceosome | Foldase protein prsA 1 (EC | 0.05 | 2.26 | 1.17 | 0.60 | 0.84 | -0.25 |
| Starch and sucrose metabolism | Alpha, alpha-phosphotrehalase (EC 3.2.1.93) | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Starch and sucrose metabolism | Alpha, alpha-phosphotrehalase | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Starch and sucrose metabolism | Maltose phosphorylase (EC | 1.00 | 1.00 | 0.00 | 0.03 | 2.33 | 1.22 |
| Starch and sucrose metabolism | Beta-phospho-glucomutase | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Starch and sucrose metabolism | Beta-phospho-glucomutase | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |

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| Starch and sucrose metabolism | Neopullulanase | 1.00 | 1.00 | 0.00 | 0.00 | 2.60 | 1.38 |
| Streptomycin biosynthesis | dTDP-glucose 4,6-dehydratase (EC 4.2.1.46) | 0.05 | 1.26 | 0.33 | 0.01 | 1.70 | 0.76 |
| Streptomycin biosynthesis | Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24) | 0.04 | 1.97 | 0.98 | 0.00 | 2.29 | 1.19 |
| Streptomycin biosynthesis | dTDP-glucose 4,6-dehydratase (EC 4.2.1.46) | 0.03 | 1.45 | 0.53 | 0.01 | 1.84 | 0.88 |
| Streptomycin biosynthesis | dTDP-glucose 4,6-dehydratase (EC 4.2.1.46) | 0.04 | 1.45 | 0.53 | 0.01 | 1.78 | 0.84 |
| Streptomycin biosynthesis | dTDP-glucose 4,6-dehydratase (EC 4.2.1.46) | 0.01 | 1.56 | 0.64 | 0.02 | 1.77 | 0.82 |
| Streptomycin biosynthesis | dTDP-4-dehydrorhamnose reductase (EC 1.1.1.133) | 0.01 | 1.40 | 0.49 | 0.22 | 1.32 | 0.40 |
| Streptomycin biosynthesis | dTDP-4-dehydrorhamnose reductase | 0.01 | 1.40 | 0.49 | 0.22 | 1.32 | 0.40 |
| Streptomycin biosynthesis | Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24) | 0.05 | 2.01 | 1.01 | 0.00 | 2.42 | 1.27 |
| Streptomycin biosynthesis | Glucose-1-phosphate thymidylyltransferase (EC 2.7.7.24) | 0.11 | 1.95 | 0.96 | 0.01 | 2.22 | 1.15 |
| Streptomycin biosynthesis | dTDP-glucose 4,6-dehydratase (EC 4.2.1.46) | 0.02 | 1.44 | 0.53 | 0.02 | 1.71 | 0.78 |
| Streptomycin biosynthesis | Inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase 2 (EC 1.1.1.18) (EC 1.1.1.n6) (Myo-inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase 2) | 1.00 | 1.00 | 0.00 | 0.00 | 9.54 | 3.25 |
| Streptomycin biosynthesis | Inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase 3 (EC 1.1.1.18) (EC 1.1.1.n6) (Myo-inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase 3) | 1.00 | 1.00 | 0.00 | 0.00 | 9.27 | 3.21 |
| Streptomycin biosynthesis | Inositol dehydrogenase | 1.00 | 1.00 | 0.00 | 0.00 | 14.08 | 3.82 |
| Streptomycin biosynthesis | Inositol dehydrogenase | 1.00 | 1.00 | 0.00 | 0.00 | 16.21 | 4.02 |
| Streptomycin biosynthesis | Inositol dehydrogenase | 1.00 | 1.00 | 0.00 | 0.00 | 16.75 | 4.07 |

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| Streptomycin biosynthesis | Inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase (EC 1.1.1.18) (EC 1.1.1.n6) (Myo-inositol 2-dehydrogenase/D-chiro-inositol 3-dehydrogenase) | 1.00 | 1.00 | 0.00 | 0.00 | 9.54 | 3.25 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ | 0.02 | 0.42 | -1.25 | 0.00 | 0.36 | -1.47 |
| Stress protein | ATP-dependent clp protease ATP-binding subunit | 0.03 | 0.45 | -1.15 | 0.00 | 0.39 | -1.37 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ | 0.02 | 0.43 | -1.22 | 0.00 | 0.36 | -1.46 |
| Stress protein | ATPase with chaperone activity, ATP-binding subunit | 0.03 | 0.46 | -1.13 | 0.00 | 0.39 | -1.37 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ | 0.00 | 0.53 | -0.92 | 0.00 | 0.38 | -1.38 |
| Stress protein | ATP-dependent Clp protease, ATP-binding subunit ClpC | 0.00 | 0.53 | -0.92 | 0.00 | 0.38 | -1.38 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ | 0.00 | 0.53 | -0.92 | 0.00 | 0.38 | -1.38 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ | 0.00 | 0.22 | -2.16 | 0.01 | 0.34 | -1.54 |
| Stress protein | ClpL | 0.00 | 0.22 | -2.16 | 0.01 | 0.34 | -1.54 |
| Stress protein | ATP-binding subunit of Clp protease and DnaK/DnaJ | 0.00 | 0.27 | -1.87 | 0.00 | 0.27 | -1.87 |
| stress protein | Similar to universal stress protein, UspA family | 0.05 | 0.67 | -0.57 | 0.19 | 0.73 | -0.45 |
| Stress protein | Chaperone ClpB | 0.03 | 0.58 | -0.80 | 0.10 | 0.68 | -0.55 |
| Stress protein | Protein grpE (HSP-70 cofactor) | 0.04 | 0.54 | -0.89 | 0.25 | 0.72 | -0.47 |
| Stress protein | Chaperone ClpB | 0.03 | 0.58 | -0.80 | 0.10 | 0.68 | -0.55 |
| Stress protein | ATP-dependent RNA helicase | 0.03 | 3.93 | 1.98 | 1.00 | 1.00 | 0.00 |
| Stress protein | ATP-dependent Clp protease ATP-binding subunit ClpX | 0.09 | 1.66 | 0.73 | 0.01 | 0.34 | -1.54 |
| Stress protein | Membrane protease subunit, stomatin/prohibitin family | 0.70 | 1.36 | 0.45 | 0.00 | 0.27 | -1.87 |
| Synthesis and degradation of ketone bodies | Hydroxymethylglutaryl-CoA synthase (EC 2.3.3.10) | 0.03 | 2.33 | 1.22 | 0.00 | 6.87 | 2.78 |

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| Synthesis and degradation of ketone bodies | Hydroxymethylglutaryl-CoA synthase (EC 2.3.3.10) | 0.03 | 2.60 | 1.38 | 0.00 | 7.94 | 2.99 |
| Synthesis and degradation of ketone bodies | 3-hydroxy-3-methylglutaryl CoA synthase | 0.03 | 2.60 | 1.38 | 0.00 | 7.40 | 2.89 |
| Transcription | Transcription antitermination protein nusG | 0.04 | 1.84 | 0.88 | 0.81 | 1.19 | 0.25 |
| Transcription factors | Putative transcriptional regulator | 0.02 | 0.73 | -0.45 | 0.54 | 0.84 | -0.25 |
| Transcription factors | Transcriptional regulator | 0.03 | 2.07 | 1.05 | 1.00 | 1.00 | 0.00 |
| Transcription factors | Transcriptional regulator, MarR family | 1.00 | 1.53 | 0.62 | 0.00 | 2.87 | 1.52 |
| Transcription factors | Transcriptional regulator, marR family | 1.00 | 1.53 | 0.62 | 0.00 | 2.87 | 1.52 |
| Transcription factors | Transcriptional regulator, MarR family | 1.00 | 1.27 | 0.34 | 0.05 | 1.80 | 0.85 |
| Translation | Sex pheromone cAD1 | 0.50 | 1.19 | 0.25 | 0.01 | 0.33 | -1.62 |
| Translation Factors | Tuf (Fragment) | 0.90 | 0.98 | -0.03 | 0.03 | 1.34 | 0.42 |
| Translation Factors | Tuf (Fragment) | 0.86 | 1.13 | 0.17 | 0.05 | 1.44 | 0.53 |
| transport | Maltose ABC transporter substrate binding protein | 0.00 | 3.08 | 1.62 | 0.00 | 2.87 | 1.52 |
| transport | ABC-type sugar transport system, periplasmic component | 0.00 | 2.59 | 1.37 | 0.00 | 2.59 | 1.37 |
| transport | RbsB (Ribose ABC transporter) (Ribose-binding protein) | 0.00 | 2.60 | 1.38 | 0.00 | 2.60 | 1.38 |
| transport | ABC-type sugar transport system, periplasmic component | 0.00 | 2.60 | 1.38 | 0.00 | 2.60 | 1.38 |
| transport | ABC-type oligopeptide transport system, periplasmic component | 0.00 | 0.34 | -1.54 | 0.00 | 0.03 | -5.30 |
| transport | ABC transporter, oligopeptide-binding protein (Oligopeptide/dipeptide ABC transporter, oligopeptide/dipeptide-binding protein) | 0.00 | 0.38 | -1.41 | 0.00 | 0.02 | -5.45 |
| transport | ABC-type oligopeptide transport system, periplasmic component | 0.00 | 0.37 | -1.44 | 0.00 | 0.03 | -5.08 |
| transport | ABC-type uncharacterized transport system, ATPase | 0.03 | 2.86 | 1.52 | 0.08 | 0.53 | -0.90 |

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| transport | ABC-type uncharacterized transport system, ATPase | 0.03 | 2.77 | 1.47 | 0.08 | 0.53 | -0.90 |
| transport | ABC-type uncharacterized transport system, ATPase | 0.03 | 2.86 | 1.52 | 0.08 | 0.53 | -0.90 |
| transport | ABC-type proline/glycine betaine transport system, ATPase component | 0.02 | 3.67 | 1.87 | 0.19 | 1.80 | 0.85 |
| transport | Glycine betaine/carnitine/choline ABC transporter, ATP-binding protein | 0.02 | 3.67 | 1.87 | 0.19 | 1.80 | 0.85 |
| transport | Cell-division associated ABC transporter, ATP binding FtsE subunit | 0.03 | 3.93 | 1.98 | 0.19 | 1.53 | 0.62 |
| transport | Carbohydrate ABC transporter substrate-binding protein, CUT1 family | 0.28 | 1.07 | 0.10 | 0.05 | 0.69 | -0.54 |
| transport | ABC-type oligopeptide transport system, periplasmic component | 0.65 | 0.92 | -0.12 | 0.05 | 0.57 | -0.80 |
| transport | Oligopeptide ABC trasporter substrate binding protein | 0.65 | 0.92 | -0.12 | 0.05 | 0.57 | -0.80 |
| transport | ABC-type oligopeptide transport system, periplasmic component | 0.35 | 0.87 | -0.20 | 0.00 | 0.41 | -1.28 |
| transport | Multiple sugar ABC transporter, ATP-binding protein | 0.17 | 2.33 | 1.22 | 0.03 | 2.87 | 1.52 |
| transport | Multiple sugar ABC transporter ATPase component | 0.17 | 2.07 | 1.05 | 0.03 | 2.87 | 1.52 |
| transport | Glutamine transport ATP-binding protein | 0.70 | 1.39 | 0.47 | 0.02 | 0.48 | -1.05 |
| transport | ABC transporter related | 0.18 | 3.13 | 1.65 | 0.04 | 1.80 | 0.85 |
| transport | ABC-type antimicrobial peptide transport system, ATPase component | 0.17 | 3.13 | 1.65 | 0.04 | 1.80 | 0.85 |
| transport | ATP binding protein | 0.37 | 0.43 | -1.22 | 0.03 | 0.43 | -1.22 |
| Two-component system | Citrate lyase acyl carrier protein (Citrate lyase gamma chain) | 0.93 | 1.10 | 0.14 | 0.00 | 2.42 | 1.27 |

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| Two-component system | Citrate lyase acyl carrier protein (Citrate lyase gamma chain) | 0.93 | 1.10 | 0.14 | 0.00 | 2.42 | 1.27 |
| Tyrosine metabolism | Aryl-alcohol dehydrogenase related enzyme | 0.03 | 0.61 | -0.72 | 0.27 | 0.85 | -0.24 |
| Ubiquinone and other terpenoid-quinone biosynthesis | Glyoxalase/bleomycin resistance protein/dioxygenase | 0.03 | 0.48 | -1.05 | 0.02 | 0.48 | -1.05 |
| Valine, leucine and isoleucine degradation | Branched-chain-amino-acid aminotransferase (EC 2.6.1.42) | 0.01 | 0.46 | -1.12 | 0.00 | 0.32 | -1.67 |
| Valine, leucine and isoleucine degradation | Branched-chain-amino-acid aminotransferase (EC 2.6.1.42) | 0.01 | 0.44 | -1.17 | 0.00 | 0.30 | -1.72 |
| Valine, leucine and isoleucine degradation | Branched-chain-amino-acid aminotransferase (EC 2.6.1.42) | 0.01 | 0.46 | -1.12 | 0.00 | 0.32 | -1.67 |

# Appendix B

## Growth of Lactobacillus casei GCRL163 on Tween 80 and impact on low pH tolerance

Table B1: Proteomics data of *Lactobacillus casei* GCRL163 in modified buffered MRS broth supplemetation with Tween 80 or citrate and Tween 80.

**CI and TW = Citrate and Tween 80**

**TW = Tween 80**

| Protein name | Gene/protein symbol | Functional Class | Function | CI and TW | TW | Log2 ratio | *p* -value |
|---|---|---|---|---|---|---|---|
| D-alanine--D-alanine ligase | Ddl | Cell wall biogenesis | late peptidoglycan biosynthesis | 8.04893697 | 2.850348799 | -1.50 | 0.01 |
| glycosyltransferase family protein | | Cell wall biogenesis | teichoic acid decoration? | 2.29591887 | 0.5 | -2.20 | 0.01 |
| dolichyl-phosphate-mannose-protein mannosyltransferase | | Cell wall biogenesis | exopolysccharide/teichoic acid biosynthesis? | 0.852074763 | 1.921063995 | 1.17 | 0.04 |
| D-alanine-poly(phosphoribitol) ligase subunit 2 | DltC | Cell wall biogenesis | teichoic acid D-alanylation | 0.5 | 1.921063995 | 1.94 | 0.00 |
| 1,2-diacylglycerol 3-glucosyltransferase | WcaL | Cell wall biogenesis | teichoic acid biosynthesis | 3.75214668 | 1.440675914 | -1.38 | 0.04 |
| phosphoglucosamine mutase | GlmM | Cell wall biogenesis | early peptidoglycan biosynthesis | 13.82672247 | 7.124931892 | -0.96 | 0.00 |
| glucosamine--fructose-6-phosphate aminotransferase | GlmS | Cell wall biogenesis | early peptidoglycan biosynthesis | 159.8947584 | 23.59747373 | -2.76 | 0.00 |
| UDP-N-acetylmuramate--L-alanine ligase | MurC | Cell wall biogenesis | late peptidoglycan biosynthesis | 1.943844108 | 0.5 | -1.96 | 0.02 |
| dTDP-glucose 4,6-dehydratase | RmlB | Cell wall biogenesis | teichoic acid decoration/exopolysaccharide biosynthesis? | 37.90820589 | 27.51160701 | -0.46 | 0.01 |
| dTDP-4-dehydrorhamnose 3,5-epimerase | | Cell wall biogenesis | teichoic acid decoration | 14.52926595 | 6.648898381 | -1.13 | 0.00 |
| D-ribitol-5-phosphate cytidylyltransferase | TarI | Cell wall biogenesis | teichoic acid biosynthesis | 26.00136314 | 8.989528666 | -1.53 | 0.03 |
| UDP-glucose 4-epimerase | GalE2 | Cell wall biogenesis | teichoic acid decoration | 5.876978958 | 0.5 | -3.56 | 0.01 |
| glycosyltransferase, YqgM family | | Cell wall biogenesis | exopolysaccharide biosynthesis | 0.5 | 1.921063995 | 1.94 | 0.00 |
| glucosamine-1-phosphate N-acetyltransferase / UDP-N-acetylglucosamine | GlmU | Cell wall biogenesis | early peptidoglycan biosynthesis | 3.752949705 | 1.456421592 | -1.37 | 0.05 |

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|---|---|---|---|---|---|---|---|
| cell wall integrity sensing response regulator CesR | CesR | Signal transduction | cell wall alteration signalling | 3.38849124 | 0.5 | -2.76 | 0.00 |
| two-component system response regulator | | Signal transduction | unknown signal transduction | 0.5 | 2.866094477 | 2.52 | 0.01 |
| response regulator ArlR | ArlR | Signal transduction | oxidative stress sensing? | 12.7217664 | 3.342127989 | -1.93 | 0.00 |
| universal stress protein, UspA superfamily | | Signal transduction | stress regulation? | 4.456296205 | 0.98038808 | -2.18 | 0.00 |
| two-component system response regulator | | Signal transduction | unknown signal transduction | 2.308302572 | 4.287158472 | 0.89 | 0.03 |
| ATP synthase subunit alpha | AtpA | Membrane bioenergetics | ATP synthesis, H+ extrusion | 8.049739995 | 14.67479402 | 0.87 | 0.04 |
| ATP synthase subunit beta | AtpD | Membrane bioenergetics | ATP synthesis, H+ extrusion | 16.67886563 | 20.875284 | 0.32 | 0.02 |
| manganese-dependent inorganic pyrophosphatase | PpaC | Membrane bioenergetics | ATP synthesis | 11.67792582 | 7.15206868 | -0.71 | 0.04 |
| quinone-oxidoreductase | YogA | Membrane bioenergetics | electron transport | 0.5 | 2.401452075 | 2.26 | 0.02 |
| cytochrome D ubiquinol oxidase subunit II | CydB | Membrane bioenergetics | electron transport | 1.580188667 | 0.5 | -1.66 | 0.00 |
| Protein translocase subunit SecA | SecA | Protein export | protein secretion: Sec system (trafficking) | 0.864458465 | 3.806770391 | 2.14 | 0.01 |
| DNA translocase FtsK | FtsK | Cytokinesis | cell division (chromosomal segegration) | 0.5 | 2.397097506 | 2.26 | 0.02 |
| cell division protein DivIB | DivIVB | Cytokinesis | cell division; divisome complex | 1.580188667 | 0.5 | -1.66 | 0.00 |
| site-specific tyrosine recombinase | XerS | Cytokinesis | cell division (chromosomal segegration) | 0.5 | 1.921063995 | 1.94 | 0.00 |
| WxL domain cell surface protein with a lectin binding domain | | Cell surface proteins/internalins | unknown function | 0.852074763 | 2.866094477 | 1.75 | 0.03 |
| WxL domain cell surface protein with a lectin binding domain | | Cell surface proteins/internalins | unknown function | 0.5 | 2.877485587 | 2.52 | 0.01 |
| cell wall protein with leucine repeat domains | | Cell surface proteins/internalins | adhesion? | 0.5 | 1.921063995 | 1.94 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| aspartate-semialdehyde dehydrogenase | Asd | Amino acid-related metabolism | L-threonine biosynthesis; L-lysine biosynthesis; L-methionine biosynthesis | 1.943844108 | 0.5 | -1.96 | 0.02 |
| pyroglutamyl peptidase | Pcp | Amino acid-related metabolism | aquisition of amino acids | 4.105024467 | 6.66028949 | 0.70 | 0.02 |
| oligoendopeptidase F | YjbG | Amino acid-related metabolism | peptidase for amino acid acquistion | 6.969551327 | 2.873131018 | -1.28 | 0.02 |
| cell-envelope associated proteinase | | Amino acid-related metabolism | peptidase for amino acid acquistion | 1.944647132 | 5.723968144 | 1.56 | 0.02 |
| aminopeptidase N | PepN | Amino acid-related metabolism | peptidase for amino acid acquistion | 4.821557695 | 1.445030483 | -1.74 | 0.01 |
| oligoendopeptidase F | YjbG2 | Amino acid-related metabolism | peptidase for amino acid acquistion | 11.67953187 | 1.936809673 | -2.59 | 0.00 |
| beta-Ala-Xaa dipeptidase | PepV | Amino acid-related metabolism | peptidase for amino acid acquistion | 8.41259241 | 15.6583524 | 0.90 | 0.02 |
| cystathionine beta-lyase PatB | PatB2 | Amino acid-related metabolism | L-methionine biosynthesis | 5.900140313 | 1.916709426 | -1.62 | 0.02 |
| oligoendopeptidase F | PepF | Amino acid-related metabolism | peptidase for amino acid acquistion | 19.5681599 | 28.45395552 | 0.54 | 0.02 |
| serine hydroxymethyltransferase | GlyA | Amino acid-related metabolism | glycine biosynthesis; folate biosynthesis; one carbon pool by folate | 8.7778539 | 3.346482558 | -1.39 | 0.00 |
| histidinol dehydrogenase | HisD | Amino acid-related metabolism | Histidine biosynthesis | 0.5 | 2.397097506 | 2.26 | 0.02 |
| neutral endopeptidase | PepO2 | Amino acid-related metabolism | peptidase for amino acid acquistion | 22.09138965 | 13.28086681 | -0.73 | 0.01 |
| PepP_like Xaa-Pro aminopeptidase, APP_like family | YghT | Amino acid-related metabolism | peptidase for amino acid acquistion | 0.864458465 | 1.921063995 | 1.15 | 0.04 |
| glutamine synthetase | GlnA | Amino acid-related metabolism | L-glutamine biosynthesis | 59.19705558 | 35.57334857 | -0.73 | 0.00 |
| PII-type proteinase | | Amino acid-related metabolism | protein degradation (casein) | 1.216533227 | 8.527568236 | 2.81 | 0.02 |
| succinate-semialdehyde dehydrogenase [NADP(+)] | GabD | Amino acid-related metabolism | succinate/GABA/aldehyde metabolism? | 26.4484924 | 36.98570343 | 0.48 | 0.02 |
| phosphopentomutase | DeoB | Nucleic acid/nucleotide metabolism | (deoxy)ribose 1-phosphate biosynthesis | 7.708442885 | 0.5 | -3.95 | 0.00 |
| purine nucleoside phosphorylase | DeoD | Nucleic acid/nucleotide metabolism | purine nucleoside interconversion/salvage | 4.807567943 | 0.5 | -3.27 | 0.02 |

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|---|---|---|---|---|---|---|---|
| pyrimidine nucleotidase, HAD_like superfamily | YfnB | Nucleic acid/nucleotide metabolism | detox. noncanonical pyrimidine nucleotides | 1.580188667 | 0.5 | -1.66 | 0.00 |
| Phosphoribosylaminoimidazole carboxylase ATPase subunit | PurK | Nucleic acid/nucleotide metabolism | purine biosynthesis | 0.86365544 | 1.921063995 | 1.15 | 0.04 |
| nucleoside deaminase family protein | | Nucleic acid/nucleotide metabolism | purine interconversion/salvage | 3.38849124 | 1.440675914 | -1.23 | 0.03 |
| thymidylate synthase | ThyA | Nucleic acid/nucleotide metabolism | dTMP biosynthesis | 7.344787445 | 1.921063995 | -1.93 | 0.00 |
| ribonucleoside-diphosphate reductase subunit alpha 2 | NrdE2 | Nucleic acid/nucleotide metabolism | deoxynucleotide biosynthesis for DNA | 5.173632457 | 10.88495358 | 1.07 | 0.04 |
| guanylate kinase | Gmk | Nucleic acid/nucleotide metabolism | dGDP/GDP biosynthesis | 1.943844108 | 3.342127989 | 0.78 | 0.02 |
| dihydroorotate dehydrogenase A | PyrDA | Nucleic acid/nucleotide metabolism | UMP/uridine/uracil biosynthesis | 0.5 | 2.397097506 | 2.26 | 0.02 |
| phosphoribosylamine--glycine ligase | PurD | Nucleic acid/nucleotide metabolism | purine biosynthesis | 1.944647132 | 3.342127989 | 0.78 | 0.02 |
| phosphoribosylformylglycinamidine synthase 2 | PurL | Nucleic acid/nucleotide metabolism | purine biosynthesis | 0.5 | 1.921063995 | 1.94 | 0.00 |
| thymidylate kinase | Tmk | Nucleic acid/nucleotide metabolism | dTDP biosynthesis | 0.5 | 3.326382311 | 2.73 | 0.02 |
| ribose-phosphate pyrophosphokinase 1 | Prs2 | Nucleic acid/nucleotide metabolism | purine biosynthesis | 14.88294676 | 9.483989829 | -0.65 | 0.04 |
| fatty acid hydratase/isomerase (oleate hydratase, linoleate isomerase) | Sph | Lipid-related metabolism | fatty acid degradation or detoxification? | 22.0897836 | 2.870449046 | -2.94 | 0.00 |
| phosphatidylglycerophosphatase A | | Lipid-related metabolism | phospholipid degradation | 3.376107538 | 0.5 | -2.76 | 0.00 |
| acetyl-CoA carboxylase biotin carboxylase subunit | AccC | Lipid-related metabolism | fatty acid biosynthesis | 0.5 | 5.250616605 | 3.39 | 0.02 |
| acetyl-CoA carboxylase biotin carboxyl carrier protein | AccB | Lipid-related metabolism | fatty acid biosynthesis | 0.5 | 9.978450991 | 4.32 | 0.00 |
| 3-oxoacyl-ACP synthase I/II | FabF | Lipid-related metabolism | fatty acid biosynthesis | 0.864458465 | 13.77700057 | 3.99 | 0.00 |
| 3-oxoacyl-[acyl-carrier-protein] reductase 4 | Bkr4 | Lipid-related metabolism | fatty acid biosynthesis | 0.5 | 5.220797845 | 3.38 | 0.05 |
| enoyl-[acyl-carrier protein] reductase II | FabK | Lipid-related metabolism | fatty acid biosynthesis | 1.216533227 | 31.78183553 | 4.71 | 0.00 |

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|---|---|---|---|---|---|---|---|
| acyl carrier protein | AcpP2 | Lipid-related metabolism | fatty acid biosynthesis | 0.5 | 19.46561112 | 5.28 | 0.00 |
| 3-oxoacyl-ACP synthase III | FabH | Lipid-related metabolism | fatty acid biosynthesis | 0.5 | 5.715259007 | 3.51 | 0.01 |
| pyridoxine 5'-phosphate oxidase-like family protein | | Cofactor-related metabolism | vitamin B6 metabolism? | 4.092640765 | 10.42734772 | 1.35 | 0.01 |
| Hydroxymethylpyrimidine/phospho methylpyrimidine kinase | ThiD2 | Cofactor-related metabolism | thiamine biosynthesis | 4.456296205 | 0.5 | -3.16 | 0.00 |
| S-adenosylmethionine synthase | MetK | Cofactor-related metabolism | S-adenosylmethionine biosynthesis | 8.037356293 | 2.381351828 | -1.75 | 0.01 |
| cysteine desulfurase (tRNA sulfurtransferase) | IscS | Cofactor-related metabolism | tRNA modification; iron-cluster assembly; thiamine biosynthesis | 0.5 | 1.921063995 | 1.94 | 0.00 |
| cysteine desulfurase IscS 1 | IscS | Cofactor-related metabolism | tRNA modification; iron-cluster assembly; thiamine biosynthesis | 3.024032775 | 0.98038808 | -1.63 | 0.03 |
| phosphopantetheine adenylyltransferase | CoaD | Cofactor-related metabolism | pantothenate/CoA biosynthesis | 0.5 | 1.921063995 | 1.94 | 0.00 |
| 2-dehydropantoate 2-reductase | PanE | Cofactor-related metabolism | pantothenate/CoA biosynthesis | 2.309105597 | 13.7743186 | 2.58 | 0.00 |
| formate--tetrahydrofolate ligase | Fhs | Cofactor-related metabolism | one carbon pool by folate | 6.981132005 | 2.85738534 | -1.29 | 0.03 |
| lipoate-protein ligase LplJ | LplJ | Cofactor-related metabolism | lipoate metabolism | 10.19853665 | 0.964642402 | -3.40 | 0.00 |
| riboflavin kinase/FMN adenylyltransferase | RibC | Cofactor-related metabolism | riboflavin/flavin mononucleotide metabolism | 0.5 | 2.877485587 | 2.52 | 0.01 |
| NH(3)-dependent NAD(+) synthetase | NadE | Cofactor-related metabolism | NAD(P)H biosynthesis | 7.70924591 | 4.282803903 | -0.85 | 0.01 |
| thiamine biosynthesis lipoprotein | ApbE | Cofactor-related metabolism | thiamine biosynthesis | 1.93226343 | 0.5 | -1.95 | 0.01 |
| glutathione reductase | Gor | Cofactor-related metabolism | glutathione biosynthesis | 0.852074763 | 1.921063995 | 1.17 | 0.04 |
| DNA gyrase subunit B | GyrA | DNA replication-related | DNA topological change | 3.376910562 | 1.456421592 | -1.21 | 0.03 |
| single-stranded DNA-binding protein 2 | Ssb2 | DNA replication-related | DNA replication initiation; DNA repair (homologous recombination) | 1.66709567 | 2.519819523 | 0.60 | 0.00 |

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|---|---|---|---|---|---|---|---|
| DNA-binding protein HU | Hup/Hbs | DNA replication-related | DNA topology change | 156.4339849 | 80.42327846 | -0.96 | 0.00 |
| DNA primase | DnaG | DNA replication-related | DNA replication initiation | 0.852074763 | 4.287158472 | 2.33 | 0.00 |
| DNA polymerase III alpha subunit | PolC | DNA replication-related | DNA replication elongation | 1.228113905 | 3.342127989 | 1.44 | 0.00 |
| Holliday junction resolvasome, helicase subunit | RuvB | DNA repair/recombination | DNA repair (homologous recombination) | 0.5 | 2.385706397 | 2.25 | 0.01 |
| recombination and DNA strand exchange inhibitor | MutS2 | DNA repair/recombination | DNA repair (mismatch) | 0.5 | 1.921063995 | 1.94 | 0.00 |
| adenine-specific DNA methylase | YtxK | DNA repair/recombination | DNA repair-related | 3.728985325 | 0.976033512 | -1.93 | 0.02 |
| ATP-dependent helicase/deoxyribonuclease subunit B | AddB | DNA repair/recombination | DNA repair (homologous recombination; SOS response) | 0.5 | 1.921063995 | 1.94 | 0.00 |
| ATP-dependent DNA helicase RecG | RecG | DNA repair/recombination | DNA repair (homologous recombination) | 0.5 | 2.401452075 | 2.26 | 0.02 |
| recombination and DNA strand exchange inhibitor | MutS | DNA repair/recombination | DNA repair (mismatch) | 0.86365544 | 1.921063995 | 1.15 | 0.04 |
| ribonuclease Z | Rnz | tRNA/Ribosome assembly/processing | tRNA processing; RNA degradation | 0.5 | 2.385706397 | 2.25 | 0.01 |
| ribosome biogenesis GTP-binding protein | Der | tRNA/Ribosome assembly/processing | ribosome processing (SSU) | 0.864458465 | 2.866094477 | 1.73 | 0.03 |
| RNA binding protein, S1_like superfamily | YabR | tRNA/Ribosome assembly/processing | unknown ribosome-associated function | 1.944647132 | 9.026383967 | 2.21 | 0.00 |
| tyrosine-protein phosphatase | Ptp | Posttranslational modification | protein phosphorylation (regulation)> | 5.536484873 | 0.5 | -3.47 | 0.00 |
| ATP-dependent Clp protease proteolytic subunit | ClpP2 | Protein folding/turnover | recycling defective proteins; virulence | 3.376910562 | 1.921063995 | -0.81 | 0.01 |
| trigger factor Tig | Tig | Protein folding/turnover | nascent protein folding | 35.75780318 | 41.73363806 | 0.22 | 0.05 |
| ATP-dependent protease ATPase subunit HslU | HslU | Protein folding/turnover | recycling defective proteins | 7.697665232 | 2.854703368 | -1.43 | 0.02 |
| chaperone protein DnaK | DnaK | Protein folding/turnover | nascent protein folding; protein rescue | 27.14347032 | 52.23417533 | 0.94 | 0.02 |
| ATP-dependent Clp protease ATP-binding subunit ClpE | ClpE | Protein folding/turnover | recycling defective proteins | 2.295115846 | 9.962705313 | 2.12 | 0.01 |

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|---|---|---|---|---|---|---|---|
| ATP-dependent chaperone/Clp protease | ClpB | Protein folding/turnover | recycling defective proteins | 1.944647132 | 3.82251607 | 0.98 | 0.04 |
| class I heat-shock protein (chaperonin) small subunit | GroES | Protein folding/turnover | nascent protein folding | 3.000068395 | 15.67845265 | 2.39 | 0.00 |
| post-translocation molecular chaperone/foldase | PrsA | Protein folding/turnover | protein secretion: exported protein folding | 0.5 | 1.921063995 | 1.94 | 0.00 |
| alpha-crystallin domain heat shock protein | | Protein folding/turnover | protein disaggregation (during rapid growth) | 0.5 | 18.02880145 | 5.17 | 0.00 |
| copper ion translocating P-type ATPase | CopZ | Cell defense/detoxification | copper export | 0.5 | 1.921063995 | 1.94 | 0.00 |
| NADH peroxidase | Npr | Cell defense/detoxification | oxidative stress management | 12.73495313 | 76.82105972 | 2.59 | 0.00 |
| Cys-based peroxiredoxin, OsmC superfamily | | Cell defense/detoxification | hydroperoxide removal | 5.173632457 | 0.5 | -3.37 | 0.00 |
| Dyp-type iron-dependent peroxidase | YfeX | Cell defense/detoxification | oxidative stress management | 11.30188667 | 6.644543812 | -0.77 | 0.01 |
| glutathione peroxidase | Gpo | Cell defense/detoxification | H2O2/hydroperoxide removal | 1.943844108 | 0.5 | -1.96 | 0.02 |
| multicopper oxidase | Mco | Cell defense/detoxification | copper detoxification | 2.296721895 | 3.342127989 | 0.54 | 0.04 |
| lactaldehyde dehydrogenase/glycolaldehyde dehydrogenase | | Cell defense/detoxification | detoxification of aldehydes | 0.5 | 3.342127989 | 2.74 | 0.00 |
| maltose O-acetyltransferase family protein | Maa | Cell defense/detoxification | detoxification | 4.092640765 | 0.5 | -3.03 | 0.00 |
| phage integrase family protein | | IS elements/foreign DNA defense | integration/recombination | 0.5 | 1.921063995 | 1.94 | 0.00 |
| conjugation protein, TraG/VirD4 family | | IS elements/foreign DNA defense | conjugative transposon | 0.852074763 | 1.921063995 | 1.17 | 0.04 |
| type I restriction modification system, M subunit | HsdM | IS elements/foreign DNA defense | foreign DNA restriction | 0.5 | 4.790328771 | 3.26 | 0.04 |
| CRISPR-associated protein | Cas9/Csn1 | IS elements/foreign DNA defense | phage infection resistance | 0.864458465 | 3.342127989 | 1.95 | 0.00 |
| tRNA-binding protein, tRNA_domain_binding superfamily | YtpR | General prediction only | unknown function (tRNA-associated?) | 0.5 | 6.184255978 | 3.63 | 0.00 |
| nitroreductase family protein | | General prediction only | unknown function | 2.636412955 | 19.43411976 | 2.88 | 0.00 |

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|---|---|---|---|---|---|---|---|
| D-hydantoinase/oxoprolinase, hydantoinase superfamily | HyuA | General prediction only | unknown function | 0.5 | 2.866094477 | 2.52 | 0.01 |
| YbaB/EbfC DNA-binding family protein | | General prediction only | unknown function | 0.5 | 2.861739909 | 2.52 | 0.01 |
| flavodoxin superfamily protein | | General prediction only | unknown function | 0.5 | 1.921063995 | 1.94 | 0.00 |
| PHP superfamily protein | | General prediction only | unknown function | 1.93226343 | 3.82251607 | 0.98 | 0.04 |
| alcohol dehydrogenase, MDR superfamily | | General prediction only | unknown function | 1.580188667 | 1.921063995 | 0.28 | 0.00 |
| peptidoglycan-binding lysin | BacA | General prediction only | unknown function | 2.660377335 | 0.98038808 | -1.44 | 0.03 |
| zinc-type alcohol dehydrogenase family protein | | General prediction only | unknown function | 2.660377335 | 0.98038808 | -1.44 | 0.03 |
| AdoMet_MTases superfamily protein | | General prediction only | unknown function | 2.660377335 | 0.98038808 | -1.44 | 0.03 |
| esterase/lipase, Aes superfamily | | General prediction only | unknown function | 1.580188667 | 0.5 | -1.66 | 0.00 |
| nucleotide-binding protein, Maf_Ham1 family | Maf | General prediction only | unknown function | 1.580188667 | 0.5 | -1.66 | 0.00 |
| SAM-dependent methyltransferase | | General prediction only | unknown function | 1.943844108 | 0.5 | -1.96 | 0.02 |
| NADP-dependent oxidoreductase | | General prediction only | unknown function | 3.752949705 | 0.964642402 | -1.96 | 0.03 |
| aldo_keto_reductase superfamily protein | | General prediction only | unknown function | 28.22044689 | 7.097795105 | -1.99 | 0.00 |
| CsbD superfamily protein | | General prediction only | stress response (phosphate starvation)? | 2.308302572 | 0.5 | -2.21 | 0.01 |
| hydrolase, HAD superfamily | YidA | General prediction only | unknown function | 8.38862803 | 1.429284805 | -2.55 | 0.02 |
| hydrolase, HAD superfamily | | General prediction only | unknown function | 3.0248358 | 0.5 | -2.60 | 0.00 |
| PTS(Glucose/Mannose family) IIB | LevE/ManX | Phosphotransferase systems | glucose/mannose uptake | 4.45709923 | 1.909672885 | -1.22 | 0.04 |
| PTS(Glucose/Mannose family) IIA | LevD/ManX | Phosphotransferase systems | glucose/mannose uptake | 1.580188667 | 3.806770391 | 1.27 | 0.01 |
| PTS system beta-glucoside-specific transporter subunit IIBCA | BglP | Phosphotransferase systems | beta-glucoside uptake | 0.86365544 | 3.82251607 | 2.15 | 0.01 |
| PTS (fructose family) subunit IIABC | FruA3 | Phosphotransferase systems | fructose uptake | 0.864458465 | 2.866094477 | 1.73 | 0.03 |

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|---|---|---|---|---|---|---|---|
| phosphoenolpyruvate-protein phosphotransferase | PtsI | Phosphotransferase systems | PTS general components | 4.808370968 | 0.5 | -3.27 | 0.00 |
| phosphocarrier protein HPr | PtsH | Phosphotransferase systems | PTS general components | 97.65506156 | 77.75201712 | -0.33 | 0.01 |
| PTS system (Lactose/DACB/beta-glucoside family) IIB | CelA | Phosphotransferase systems | cellobiose uptake | 3.7281823 | 0.964642402 | -1.95 | 0.02 |
| PTS (Mannose/Fructose/Sorbose family) IIAB | ManX | Phosphotransferase systems | glucose/mannose uptake | 45.22408697 | 21.35131752 | -1.08 | 0.00 |
| PTS (Fructose/Mannitol family) subunit IIBC | | Phosphotransferase systems | mannitol uptake | 0.5 | 2.385706397 | 2.25 | 0.01 |
| ribose ABC-type transport system, substrate=binding protein | RsbB | ABC-type transporter systems | D-ribose uptake | 0.5 | 3.82251607 | 2.93 | 0.00 |
| ABC transporter ATP-binding protein YkpA | YkpA | ABC-type transporter systems | unknown transport | 0.86365544 | 2.877485587 | 1.74 | 0.03 |
| ABC-type transporter, substrate-binding protein | | ABC-type transporter systems | unknown transport | 0.5 | 4.774583093 | 3.26 | 0.01 |
| ABC transporter, ATP-binding component | | ABC-type transporter systems | unknown transport | 1.580188667 | 0.5 | -1.66 | 0.00 |
| maltose/maltodextrin ABC-type transporter, substrate binding protein | MdxE/MalE | ABC-type transporter systems | maltose/maltodextrin uptake | 13.12337597 | 4.298549581 | -1.61 | 0.00 |
| glycerol-3-phosphate ABC transporter substrate-binding protein | UgpB | ABC-type transporter systems | sugar/glycerol uptake | 15.24820825 | 3.357873667 | -2.18 | 0.00 |
| ABC-type transporter, permease component | YdbJ | ABC-type transporter systems | unknown transport | 0.5 | 1.921063995 | 1.94 | 0.00 |
| bacteriocin processing/export ABC-type transporter, permease/ATP-binding components | | ABC-type transporter systems | bacteriocin export | 0.5 | 1.921063995 | 1.94 | 0.00 |
| sugar/glycerol-3-phosphate uptake ABC-type importer, substrate-binding protein | | ABC-type transporter systems | sugar/glycerol-3-phosphate uptake? | 3.376107538 | 0.98038808 | -1.78 | 0.02 |
| polar amino acid ABC-type importer, permease component | GlnH4 | ABC-type transporter systems | polar amino acid uptake | 0.5 | 2.401452075 | 2.26 | 0.02 |

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|---|---|---|---|---|---|---|---|
| polar amino acid ABC-type importer, permease component | GlnH5 | ABC-type transporter systems | polar amino acid uptake | 0.5 | 2.385706397 | 2.25 | 0.01 |
| high affinity gluconate transporter | GntP | Other transporter proteins | gluconate uptake | 0.5 | 1.921063995 | 1.94 | 0.00 |
| MFS-type transporter | YceJ | Other transporter proteins | unknown transport | 0.864458465 | 1.921063995 | 1.15 | 0.04 |
| MFS_1 superfamily transporter | YdiM | Other transporter proteins | unknown transport | 0.5 | 1.921063995 | 1.94 | 0.00 |
| ferrous iron transport protein A | FeoA | Other transporter proteins | ferrous iron uptake | 1.580188667 | 0.5 | -1.66 | 0.00 |
| Trk-type K+ transport ATPase | YjbQ | Other transporter proteins | potassium ion uptake | 0.864458465 | 4.298549581 | 2.31 | 0.01 |
| acetate kinase | AckA | Carbohydrate-related metabolism | fermentation; end-product formation | 18.87318198 | 3.342127989 | -2.50 | 0.00 |
| 3D-(3,5/4)-trihydroxycyclohexane-1,2-dione hydrolase | IolD | Carbohydrate-related metabolism | inositol-related compound degradation | 0.5 | 2.401452075 | 2.26 | 0.02 |
| trehalose-6-phosphate hydrolase | TreA | Carbohydrate-related metabolism | trehalose catabolism | 5.899337288 | 10.9077358 | 0.89 | 0.03 |
| L-lactate dehydrogenase | | Carbohydrate-related metabolism | fermentation; end-product formation | 2.660377335 | 1.921063995 | -0.47 | 0.00 |
| glycerol-3-phosphate dehydrogenase (aerobic) | GlpD | Carbohydrate-related metabolism | glycerol catabolism | 0.852074763 | 9.502417479 | 3.48 | 0.00 |
| glycerol kinase | GlpK | Carbohydrate-related metabolism | glycerol catabolism | 0.5 | 24.18909094 | 5.60 | 0.00 |
| galactose-1-phosphate uridylyltransferase | GalT | Carbohydrate-related metabolism | galactose/tagatose catabolism | 36.93879135 | 12.82176325 | -1.53 | 0.00 |
| tagatose 1,6-diphosphate aldolase 2 | LacD2 | Carbohydrate-related metabolism | galactose/tagatose catabolism | 16.01267024 | 9.502417479 | -0.75 | 0.04 |
| Galactose-6-phosphate isomerase subunit lacB | LacB | Carbohydrate-related metabolism | lactose catabolism | 11.32665408 | 4.736055196 | -1.26 | 0.03 |
| galactose-6-phosphate isomerase subunit LacA | LacA | Carbohydrate-related metabolism | lactose catabolism | 4.481866634 | 0.5 | -3.16 | 0.04 |
| 6-phosphogluconolactonase | YwcC | Carbohydrate-related metabolism | pentose phosphate pathway | 5.549671599 | 1.456421592 | -1.93 | 0.01 |
| 1-phosphofructokinase | FruK | Carbohydrate-related metabolism | fructose catabolism | 0.864458465 | 9.049166187 | 3.39 | 0.01 |
| formate acetyltransferase | PflB | Carbohydrate-related metabolism | fermentation, end-product formation | 239.8483307 | 106.1722876 | -1.18 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| pyruvate formate-lyase activating enzyme | PflA | Carbohydrate-related metabolism | fermentation; end-product formation | 10.57377277 | 1.445030483 | -2.87 | 0.00 |
| aryl-phospho-beta-D-glucosidase | BglA | Carbohydrate-related metabolism | beta-glucoside catabolism | 4.468679907 | 1.921063995 | -1.22 | 0.00 |
| pyruvate oxidase | Pox5 | Carbohydrate-related metabolism | fermentation; end-product metabolism | 24.60705384 | 6.129982403 | -2.01 | 0.01 |
| glucose-1-phosphate adenylyltransferase | GlgD | Carbohydrate-related metabolism | starch/glycogen synthesis | 3.376107538 | 6.676035168 | 0.98 | 0.04 |
| 1,4-alpha-glucan branching enzyme | GlgB | Carbohydrate-related metabolism | starch/glycogen hydrolysis/synthesis | 1.943844108 | 0.5 | -1.96 | 0.02 |
| oligo-1,6-glucosidase | MalL | Carbohydrate-related metabolism | maltose/polysaccharide metabolism? | 0.5 | 1.921063995 | 1.94 | 0.00 |
| NADH-dependent butanol dehydrogenase A | BdhA | Carbohydrate-related metabolism | fermentation; end-product metabolism | 8.788631553 | 5.708222466 | -0.62 | 0.03 |
| aryl-phospho-beta-D-glucosidase | BglH | Carbohydrate-related metabolism | beta-glucoside catabolism | 4.45709923 | 7.129286461 | 0.68 | 0.01 |
| L-lactate dehydrogenase | Ldh | Carbohydrate-related metabolism | fermentation; end-product formation | 217.9136996 | 298.9177288 | 0.46 | 0.01 |
| tagatose 1,6-diphosphate aldolase 2 | LacD2 | Carbohydrate-related metabolism | galactose/tagatose catabolism | 75.4338246 | 90.00257684 | 0.25 | 0.01 |
| oligo-1,6-glucosidase | | Carbohydrate-related metabolism | degradation of dextrin/maltotriose | 24.25016093 | 13.30096706 | -0.87 | 0.00 |
| L-xylulose 5-phosphate 3-epimerase | SgaU | Carbohydrate-related metabolism | ascorbate utilization | 0.5 | 2.401452075 | 2.26 | 0.02 |
| myo-inositol catabolism protein IolS | IolS | Carbohydrate-related metabolism | myo-inositol catabolism | 17.04252107 | 7.616711082 | -1.16 | 0.00 |
| xylulose-5-phosphate phosphoketolase | XpaK | Central glycolytic/intermediary pathways | pentose phosphate pathway; D-xylulose catabolism | 21.37646247 | 47.82826287 | 1.16 | 0.00 |
| ribokinase | RsbK | Central glycolytic/intermediary pathways | pentose phosphate pathway | 1.580188667 | 0.5 | -1.66 | 0.00 |
| fructose-bisphosphate aldolase | Fba2 | Central glycolytic/intermediary pathways | glycolysis | 56.58312479 | 109.8545903 | 0.96 | 0.00 |
| galactose 1-epimerase, aldos_epim superfamily | GalM | Central glycolytic/intermediary pathways | galactose/tagatose catabolism | 55.97322674 | 23.68156607 | -1.24 | 0.00 |
| glucose-6-phosphate 1-dehydrogenase | Zwf | Central glycolytic/intermediary pathways | pentose phosphate pathway | 11.66473909 | 6.633152703 | -0.81 | 0.03 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| glyceraldehyde-3-phosphate dehydrogenase | Gap | Central glycolytic/intermediary pathways | glycolysis | 210.3819645 | 66.80258312 | -1.66 | 0.00 |
| phosphoglycerate kinase | Pgk | Central glycolytic/intermediary pathways | glycolysis | 17.07967218 | 114.1442559 | 2.74 | 0.00 |
| glucose-6-phosphate isomerase | Pgi | Central glycolytic/intermediary pathways | glycolysis | 56.23265608 | 46.40719887 | -0.28 | 0.04 |
| Pyruvate dehydrogenase E1 component subunit alpha | PdhA | Central glycolytic/intermediary pathways | glycolysis | 24.96830021 | 14.66340291 | -0.77 | 0.01 |
| dihydrolipoyl dehydrogenase | PdhD | Central glycolytic/intermediary pathways | glycolysis | 9.140706315 | 0.98038808 | -3.22 | 0.00 |
| pyruvate kinase | Pyk | Central glycolytic/intermediary pathways | glycolysis | 80.77686455 | 50.20574845 | -0.69 | 0.00 |
| aldose 1-epimerase | | Central glycolytic/intermediary pathways | glycolysis | 8.425779137 | 1.445030483 | -2.54 | 0.00 |
| glucokinase | GlcK | Central glycolytic/intermediary pathways | glycolysis | 22.81629145 | 44.58715716 | 0.97 | 0.01 |
| 6-phosphogluconate dehydrogenase | Gnd | Central glycolytic/intermediary pathways | pentose phosphate pathway | 62.08233472 | 41.23314973 | -0.59 | 0.00 |
| fructose-1,6-bisphosphatase class 3 | Fpb | Central glycolytic/intermediary pathways | glycolysis | 7.722432637 | 0.5 | -3.95 | 0.01 |
| 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase | GpmA2 | Central glycolytic/intermediary pathways | glycolysis | 114.7050244 | 75.31322141 | -0.61 | 0.00 |
| pyruvate, phosphate dikinase | PpdK | Central glycolytic/intermediary pathways | glycolysis | 58.09049346 | 5.225152413 | -3.47 | 0.00 |
| fumarate reductase flavoprotein subunit | IfcA | Tricarboxylic acid pathway | fermentation; TCA cycle | 0.86365544 | 3.326382311 | 1.95 | 0.05 |
| pyruvate carboxylase | Pyc | Tricarboxylic acid pathway | TCA cycle | 6.981132005 | 0.98038808 | -2.83 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| oxaloacetate decarboxylase, alpha subunit | OadA | Tricarboxylic acid pathway | citrate utilization | 45.09188137 | 6.157119191 | -2.87 | 0.00 |
| apo-citrate lyase phosphoribosyl-dephospho-CoA transferase | CitX | Tricarboxylic acid pathway | citrate utilization | 3.389294264 | 0.5 | -2.76 | 0.02 |
| citrate lyase alpha subunit | CitF | Tricarboxylic acid pathway | citrate utilization | 94.01047691 | 23.64303817 | -1.99 | 0.00 |
| citrate lyase beta subunit | CitE | Tricarboxylic acid pathway | citrate utilization | 55.57482927 | 5.200697598 | -3.42 | 0.00 |
| citrate lyase acyl carrier protein | CitD | Tricarboxylic acid pathway | citrate utilization | 6.264598778 | 3.806770391 | -0.72 | 0.01 |
| oxaloacetate decarboxylase biotin carrier protein | OadH | Tricarboxylic acid pathway | citrate utilization | 4.105024467 | 0.5 | -3.04 | 0.00 |
| DNA-directed RNA polymerase subunit alpha | RpoA | RNA polymerase | RNA polymerase | 20.29386473 | 12.82493355 | -0.66 | 0.01 |
| DNA-directed RNA polymerase subunit beta' | RpoC | RNA polymerase | RNA polymerase | 27.82044337 | 18.98689563 | -0.55 | 0.04 |
| DNA-directed RNA polymerase subunit beta | RpoB | RNA polymerase | RNA polymerase | 44.04723776 | 21.35399949 | -1.04 | 0.00 |
| transcriptional antiterminator with sigma54 interaction domain | | Transcriptional regulation | regulation of sugar uptake PTS system genes | 0.5 | 1.921063995 | 1.94 | 0.00 |
| HxlR family transcriptional regulator | YybR | Transcriptional regulation | regulatory role unknown | 0.5 | 1.921063995 | 1.94 | 0.00 |
| MarR family transcriptional regulator | | Transcriptional regulation | regulatory role unknown | 3.024032775 | 0.964642402 | -1.65 | 0.03 |
| catabolite control protein A | CcpA | Transcriptional regulation | regulation of catabolic enzyme coding genes | 18.13429042 | 2.381351828 | -2.93 | 0.00 |
| transcriptional regulator, HxlR family | YodB | Transcriptional regulation | regulatory role (unknown) | 1.943844108 | 0.5 | -1.96 | 0.02 |
| XRE family transcriptional regulator | | Transcriptional regulation | regulatory role unknown | 34.71476562 | 27.98931311 | -0.31 | 0.05 |
| redox (NADH)-sensing transcriptional repressor | Rex | Transcriptional regulation | regulates genes assoc. fermentation; NADH:NAD sensor | 17.38301516 | 6.633152703 | -1.39 | 0.01 |
| GntR family transcriptional regulator | | Transcriptional regulation | regulation of sugar metabolism? | 0.5 | 3.353519099 | 2.75 | 0.03 |
| bifunctional oligoribonuclease and PAP phosphatase nrnA | NrnA | RNA degradation | RNA degradation (nanoRNA) | 10.57457579 | 3.357873667 | -1.65 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cold shock protein 1 | Csp | Transcription-associated proteins | RNA chaperone | 18.18221918 | 137.9572935 | 2.92 | 0.00 |
| Cold shock-like protein CspLA | CspLA | Transcription-associated proteins | RNA chaperone | 5.196793812 | 21.35835406 | 2.04 | 0.00 |
| transcription termination factor | NusB | Transcription-associated proteins | transcription antitermination | 0.5 | 1.921063995 | 1.94 | 0.00 |
| transcription antitermination protein NusG | NusG | Transcription-associated proteins | transcription antitermination | 13.43910265 | 6.188610547 | -1.12 | 0.01 |
| | | | | | | | |
| 30S ribosomal protein S6 | RspF | Ribosomal proteins | 30S (SSU) ribosome | 15.26896053 | 35.55056635 | 1.22 | 0.00 |
| 30S ribosomal protein S14 | RpsN | Ribosomal proteins | 30S (SSU) ribosome | 2.308302572 | 0.5 | -2.21 | 0.01 |
| 30S ribosomal protein S4 | RpsD | Ribosomal proteins | 30S (SSU) ribosome | 60.52543125 | 22.74959929 | -1.41 | 0.00 |
| 30S ribosomal protein S20 | RpsT | Ribosomal proteins | 30S (SSU) ribosome | 4.081060087 | 0.98038808 | -2.06 | 0.04 |
| 30S ribosomal protein S15 | RpsO | Ribosomal proteins | 30S (SSU) ribosome | 8.41259241 | 3.322027743 | -1.34 | 0.03 |
| 30S ribosomal protein S1 | RpsA | Ribosomal proteins | 30S (SSU) ribosome | 31.10699375 | 91.83568226 | 1.56 | 0.00 |
| 30S ribosomal protein S2 | RpsB | Ribosomal proteins | 30S (SSU) ribosome | 50.51598606 | 26.52536665 | -0.93 | 0.00 |
| 50S ribosomal protein L19 | RplS | Ribosomal proteins | 50S (LSU) ribosome | 13.45148636 | 9.030738536 | -0.57 | 0.03 |
| 30S ribosomal protein S16 | RpsP | Ribosomal proteins | 30S (SSU) ribosome | 6.61667354 | 17.08812553 | 1.37 | 0.00 |
| 50S ribosomal protein L20 | RplT | Ribosomal proteins | 50S (LSU) ribosome | 4.45709923 | 0.5 | -3.16 | 0.00 |
| 50S ribosomal protein L35 | RpmI | Ribosomal proteins | 50S (LSU) ribosome | 24.58228644 | 0.5 | -5.62 | 0.00 |
| 50S ribosomal protein L7/L12 | RplL | Ribosomal proteins | 50S (LSU) ribosome | 44.03485406 | 23.15561355 | -0.93 | 0.01 |
| | | | | | | | |
| 50S ribosomal protein L1 | RplA | Ribosomal proteins | 50S (LSU) ribosome | 17.74426152 | 8.081353485 | -1.13 | 0.02 |
| 50S ribosomal protein L13 | RplM | Ribosomal proteins | 50S (LSU) ribosome | 8.060517648 | 3.826870638 | -1.07 | 0.04 |
| 50S ribosomal protein L17 | RpsQ | Ribosomal proteins | 50S (LSU) ribosome | 1.580188667 | 0.5 | -1.66 | 0.00 |
| 30S ribosomal protein S11 | RpsK | Ribosomal proteins | 30S (SSU) ribosome | 10.22169801 | 3.326382311 | -1.62 | 0.00 |
| 50S ribosomal protein L15 | RplO | Ribosomal proteins | 50S (LSU) ribosome | 6.641440945 | 10.45883907 | 0.66 | 0.05 |
| 30S ribosomal protein S5 | RpsE | Ribosomal proteins | 30S (SSU) ribosome | 5.888559635 | 12.8450338 | 1.13 | 0.02 |
| 50S ribosomal protein L18 | RplR | Ribosomal proteins | 50S (LSU) ribosome | 15.64499968 | 6.676035168 | -1.23 | 0.01 |
| 50S ribosomal protein L6 | RplF | Ribosomal proteins | 50S (LSU) ribosome | 5.173632457 | 2.401452075 | -1.11 | 0.03 |
| 30S ribosomal protein S8 | RpsH | Ribosomal proteins | 30S (SSU) ribosome | 10.58535345 | 14.72203106 | 0.48 | 0.01 |
| 30S ribosomal protein S14 type Z | RpsZ | Ribosomal proteins | 30S (SSU) ribosome | 5.549671599 | 0.976033512 | -2.51 | 0.01 |
| | | | | | | | |
| 50S ribosomal protein L24 | RpsX | Ribosomal proteins | 50S (LSU) ribosome | 1.93226343 | 0.5 | -1.95 | 0.01 |
| 30S ribosomal protein S17 | RpsQ | Ribosomal proteins | 30S (SSU) ribosome | 40.10252223 | 29.92493852 | -0.42 | 0.03 |
| 50S ribosomal protein L16 | RplP | Ribosomal proteins | 50S (LSU) ribosome | 8.04893697 | 3.326382311 | -1.27 | 0.01 |
| 30S ribosomal protein S3 | RpsC | Ribosomal proteins | 30S (SSU) ribosome | 30.04354224 | 20.85250179 | -0.53 | 0.03 |
| 50S ribosomal protein L22 | RplV | Ribosomal proteins | 50S (LSU) ribosome | 45.44911261 | 8.022725341 | -2.50 | 0.00 |
| 50S ribosomal protein L2 | RplB | Ribosomal proteins | 50S (LSU) ribosome | 56.34009428 | 29.80064569 | -0.92 | 0.00 |
| 50S ribosomal protein L23 | RplW | Ribosomal proteins | 50S (LSU) ribosome | 42.24054124 | 23.14254984 | -0.87 | 0.02 |
| 50S ribosomal protein L4 | RplD | Ribosomal proteins | 50S (LSU) ribosome | 9.11754496 | 0.976033512 | -3.22 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 30S ribosomal protein S10 | RpsJ | Ribosomal proteins | 30S (SSU) ribosome | 10.57457579 | 39.5059095 | 1.90 | 0.05 |
| 30S ribosomal protein S12 | RplL | Ribosomal proteins | 30S (SSU) ribosome | 10.5713637 | 18.51789866 | 0.81 | 0.04 |
| 50S ribosomal protein L31 | RpmE | Ribosomal proteins | 50S (LSU) ribosome | 15.2589859 | 7.117895351 | -1.10 | 0.00 |
| glutamyl-tRNA(Gln) amidotransferase subunit A | GatA | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 12.01681385 | 4.767546552 | -1.33 | 0.00 |
| aspartyl/glutamyl-tRNA amidotransferase subunit B | GatB | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 17.7590543 | 8.076998916 | -1.14 | 0.00 |
| isoleucine--tRNA ligase | IleS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 6.266204827 | 2.401452075 | -1.38 | 0.02 |
| glycine--tRNA ligase subunit beta | GlyS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 8.07290135 | 2.417197753 | -1.74 | 0.02 |
| Proline--tRNA ligase | ProS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 11.65556749 | 18.98086847 | 0.70 | 0.02 |
| threonine--tRNA ligase | ThrS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 36.14381695 | 15.19538259 | -1.25 | 0.00 |
| purine nucleoside phosphoramidase, putative | HinT2 | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 3.0248358 | 1.921063995 | -0.65 | 0.04 |
| serine--tRNA ligase | SerS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 10.23327868 | 1.925418563 | -2.41 | 0.00 |
| lysine--tRNA ligase | LysS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 6.628254218 | 1.909672885 | -1.80 | 0.01 |
| tryptophan--tRNA ligase | TrpS | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 5.161248755 | 1.456421592 | -1.83 | 0.02 |
| translation initiation factor IF-1 | InfA | Protein translation (initiation) | protein synthesis; translation initiation | 0.864458465 | 2.877485587 | 1.73 | 0.03 |
| elongation factor Tu | Tuf | Protein translation (elongation) | protein synthesis; translation elongation | 566.448913 | 864.1751828 | 0.61 | 0.00 |
| elongation factor P | Efp | Protein translation (elongation) | protein synthesis; translation elongation | 11.66473909 | 8.081353485 | -0.53 | 0.03 |
| elongation factor P | Efp | Protein translation (elongation) | protein synthesis; translation elongation | 4.45709923 | 3.342127989 | -0.42 | 0.03 |
| elongation factor G | Fus | Protein translation (elongation) | protein synthesis; translation elongation | 71.85196149 | 47.76092559 | -0.59 | 0.02 |
| GTP-and nucleic acid- binding protein | EngD | Protein translation (peptide release) | translation attenuation? | 13.47384469 | 1.909672885 | -2.82 | 0.00 |
| | | | | 98.4179175 | 46.96899732 | -1.07 | 0.00 |

**Appendix C**

**Growth phase dependent and independent acid adaptation responses in *Lactobacillus casei***

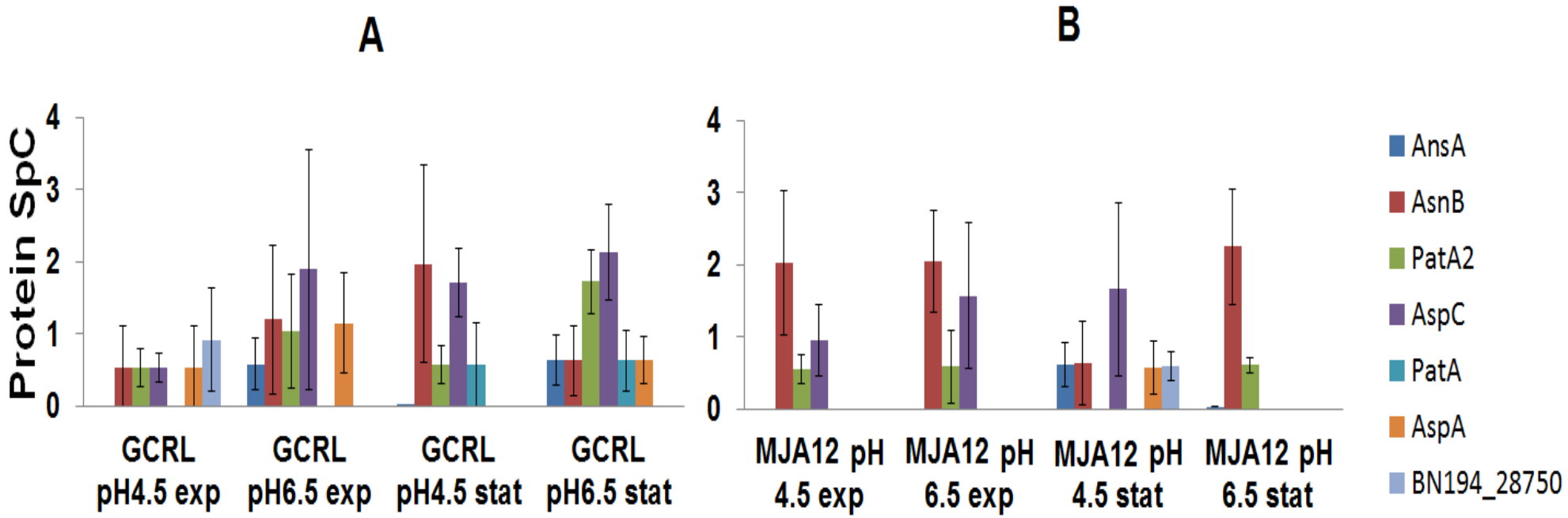


**Figure C1.** Spectral count abundance data for enzymes associated with aspartate-related metabolism in *Lb. casei* strains A) GCRL163 and B) MJA12 grown anaerobically at different pH. The SpC values are the average obtained per biological replicate based on spectra that pass filteration criteria. The error bars are the standard deviation values derived from the comparisons. Where values are absent spectra were not observed for the enymze in the given samples. Abbreviations: **exp** (exponential growth phase); **stat** (stationary growth phase). **AnsA**: L-

asparaginase, **AsnB**: asparagine synthetase, **PatA2**: aromatic amino acid aminotransferase, **AspC**: aspartate aminotransferase, **PatA**: aromatic amino acid aminotransferase, **AspA**: aspartate ammonia-lyase, **BN194_28750**: aspartate 4-decarboxylase, putative.

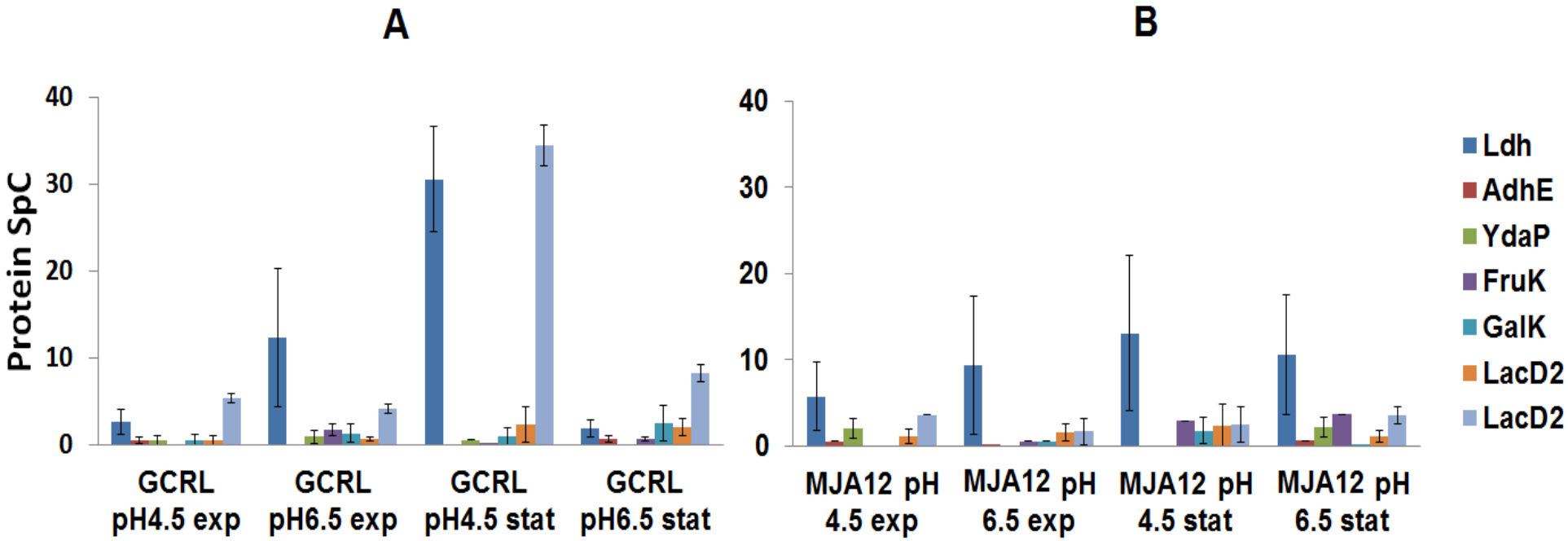


**Figure C2.** Spectral count abundance data for enzymes associated with carbohydrate fermentation in *Lb. casei* strains A) GCRL163 and B) MJA12 grown anaerobically at different pH. For other details and abbreviations see Figure S1. **Ldh**: L-lactate dehydrogenase, **AdhE**: acetaldehyde-CoA dehydrogenase/iron-dependent alcohol dehydrogenase, **YdaP**: pyruvate oxidase, **FruK**: 1-phosphofructokinase, **GalK**: galactokinase, **LacD2**: tagatose 1,6-diphosphate aldolase 2, **LacD2**: tagatose 1,6-diphosphate aldolase .

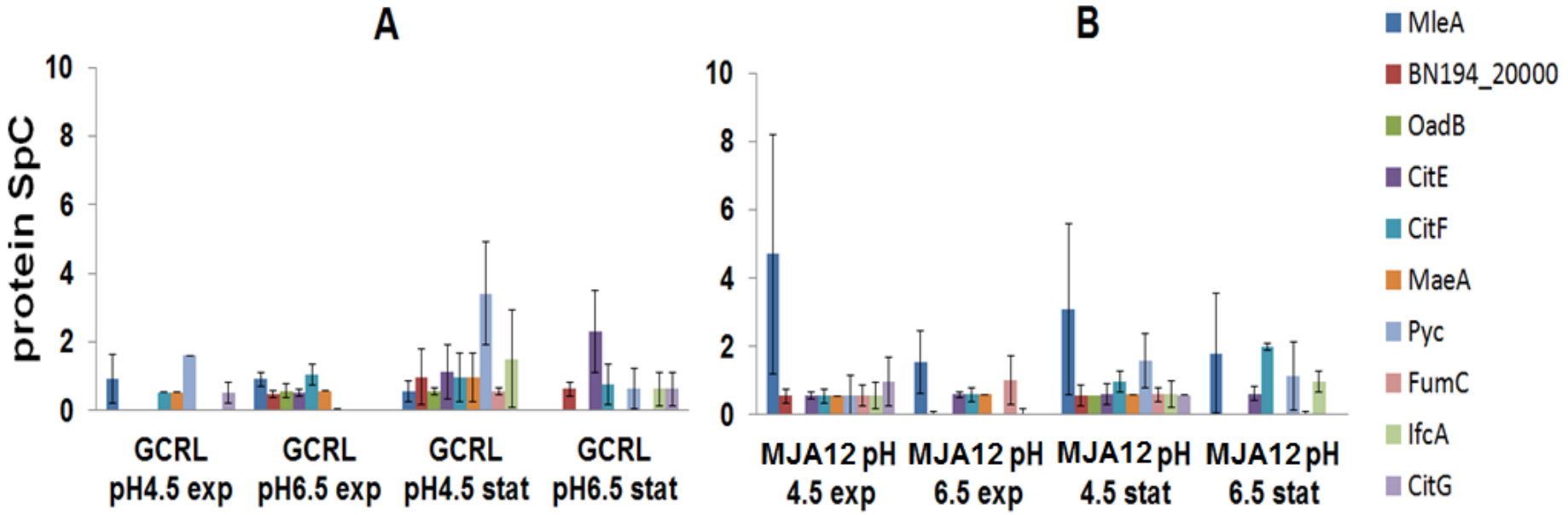


**Figure C3.** Spectral count abundance data for enzymes associated with citrate and malolactic fermentation in *Lb. casei* strains A) GCRL163 and B) MJA12 grown anaerobically at different pH. For other details and abbreviations see Figure S1. **MleA**: malate dehydrogenase, **BN194_20000**: phosphoenolpyruvate carboxykinase, **OadB**: oxaloacetate decarboxylase beta subunit, **CitE**: citrate lyase beta subunit, **CitF**: citrate lyase alpha subunit, **MaeA**: malate dehydrogenase, **Pyc**: pyruvate carboxylase, **FumC**: class II fumarate hydratase, **IfcA**: fumarate reductase flavoprotein subunit, **CitG**: 2-(5"-triphosphoribosyl)-3'-dephosphocoenzyme-A synthase.

Table C1: Proteomics data of *Lactobacillus casei* GCRL163 and MJA12 under pH 6.5 and 4.5 at exponantioal and stationary growth phases.

**GCRL = Lactobacillus casei GCRL163**
**MJA12 = Lactobacillus casei MJA12**

| | | Gene/protein symbol | GC pH4.5/pH6.5 exp | | MJA12 pH4.5/pH 6.5 exp | | GC pH4.5/pH6.5 stat | | MJA12 pH4.5/pH6.5 stat | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Log2 Fold change | TTEST | Log2 Fold change | TTEST | Log2 Fold change | TTEST | Log2 Fold change | TTEST |
| **Function** | **Protein name** | | | | | | | | | |
| 30S (SSU) ribosome | 30S ribosomal protein S5 | RpsE | -1.00 | 0.02 | 0.60 | 0.01 | 1.71 | 0.01 | 0.94 | 0.01 |
| 50S (LSU) ribosome | 50S ribosomal protein L2 | RplB | -1.08 | 0.00 | -0.26 | 0.04 | -0.65 | 0.02 | -0.31 | 0.02 |
| 50S (LSU) ribosome | 50S ribosomal protein L16 | RplP | -1.26 | 0.00 | 0.54 | 0.01 | 1.54 | 0.00 | -2.78 | 0.00 |
| adenosine/adenine/AMP biosynthesis | adenylosuccinate synthetase | PurA | 0.65 | 0.01 | -0.92 | 0.01 | 0.86 | 0.01 | 0.37 | 0.01 |
| ATP synthesis, H+ extrusion | ATP synthase subunit gamma | AtpG | -1.07 | 0.01 | -1.32 | 0.01 | -1.41 | 0.02 | 2.65 | 0.00 |
| D-alanine biosynthesis; early peptidoglycan biosynthesis; teichoic biosynthesis | alanine racemase | Alr | 1.54 | 0.01 | 1.23 | 0.03 | -1.31 | 0.02 | -0.79 | 0.02 |
| galactose/tagatose catabolism | tagatose 1,6-diphosphate aldolase 2 | LacD2 | 1.29 | 0.00 | 0.26 | 0.02 | -0.87 | 0.01 | -0.06 | 0.04 |
| glucose/mannose uptake | PTS (Mannose/Fructose/Sorbose family) IIAB | ManX | 1.01 | 0.00 | -0.58 | 0.04 | -0.84 | 0.00 | 1.10 | 0.01 |
| glycolysis | enolase | Eno | 4.35 | 0.00 | 1.29 | 0.00 | -1.58 | 0.00 | -0.66 | 0.00 |
| glycolysis | glyceraldehyde-3-phosphate dehydrogenase | Gap | 0.34 | 0.03 | 0.22 | 0.01 | -0.54 | 0.00 | -0.34 | 0.01 |
| glycolysis | 6-phosphofructokinase | Pfk | -2.15 | 0.00 | -0.73 | 0.00 | 2.52 | 0.00 | 0.48 | 0.02 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| L-glutamine biosynthesis | glutamine synthetase | GlnA | 0.26 | 0.01 | -1.43 | 0.00 | 1.21 | 0.00 | -0.52 | 0.00 |
| L-leucine/L-isoleucine /L-valine metabolism | branched-chain amino acid aminotransferase | IlvE | 1.32 | 0.02 | 2.50 | 0.00 | -1.81 | 0.00 | -2.12 | 0.00 |
| peptidase for amino acid acquistion | Proline iminopeptidase | Pip2 | 2.41 | 0.03 | 2.08 | 0.01 | -1.49 | 0.04 | -1.46 | 0.00 |
| peptidase for amino acid acquistion | aminopeptidase N | PepN | 0.91 | 0.03 | 1.12 | 0.01 | 1.92 | 0.00 | -0.39 | 0.02 |
| polar amino acid uptake | polar amino acid ABC-type exporter, ATP-binding protein | GlnQ3 | -3.77 | 0.00 | 1.83 | 0.01 | -1.98 | 0.02 | 2.32 | 0.05 |
| protein secretion: Sec system (trafficking) | Protein translocase subunit SecA | SecA | -1.47 | 0.00 | -0.60 | 0.01 | -1.11 | 0.04 | 1.59 | 0.00 |
| PTS general components | phosphocarrier protein HPr | PtsH | 0.47 | 0.00 | -0.52 | 0.00 | -1.36 | 0.00 | 0.60 | 0.02 |
| stress regulation? | universal stress protein, UspA superfamily | | 1.10 | 0.01 | -2.01 | 0.02 | -1.42 | 0.01 | 1.58 | 0.00 |
| 30S (SSU) ribosome | 30S ribosomal protein S6 | RspF | -1.41 | 0.01 | 0.99 | 0.00 | -0.82 | 0.03 | -0.26 | 0.28 |
| 50S (LSU) ribosome | 50S ribosomal protein L1 | RplA | -0.89 | 0.00 | 0.51 | 0.01 | 0.76 | 0.02 | -0.23 | 0.06 |
| 50S (LSU) ribosome | 50S ribosomal protein L21 | RplU | -0.96 | 0.01 | -0.29 | 0.05 | 1.99 | 0.02 | 0.74 | 0.37 |
| 50S (LSU) ribosome | 50S ribosomal protein L33 | RpmG | -1.74 | 0.01 | 0.86 | 0.02 | 3.03 | 0.01 | | . |

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|---|---|---|---|---|---|---|---|---|---|---|
| DNA repair (homologous recombination; SOS response) | DNA recombinase RecA | RecA | -2.24 | 0.02 | -1.90 | 0.02 | 1.06 | 0.01 | 0.46 | 0.20 |
| glycolysis | phosphoglycerate kinase | Pgk | -0.22 | 0.01 | 0.30 | 0.04 | 0.78 | 0.01 | -0.16 | 0.17 |
| oxidative stress management | NADH peroxidase | Npr | -1.50 | 0.00 | -0.90 | 0.01 | 1.24 | 0.00 | -0.27 | 0.17 |
| peptidase for amino acid acquistion | neutral endopeptidase | PepO2 | 1.65 | 0.00 | 1.14 | 0.00 | -0.54 | 0.04 | -0.11 | 0.57 |
| protein synthesis; translation initiation | translation initiation factor IF-2 | InfB | -1.64 | 0.00 | 0.63 | 0.01 | -0.39 | 0.04 | -0.02 | 0.87 |
| regulatory role (unknown) | TetR family transcriptional regulator | | -1.35 | 0.00 | 1.40 | 0.00 | 1.26 | 0.00 | -0.13 | 0.62 |
| ribosome processing (LSU); RNA chaperone | DEAD/DEAH box helicase | CshA | -2.90 | 0.00 | -0.38 | 0.03 | 0.60 | 0.01 | 0.06 | 0.48 |
| RNA polymerase | DNA-directed RNA polymerase subunit beta' | RpoC | -0.84 | 0.00 | -0.21 | 0.02 | 1.24 | 0.00 | -0.01 | 0.91 |
| 30S (SSU) ribosome | 30S ribosomal protein S16 | RpsP | -0.46 | 0.00 | 0.95 | 0.00 | 0.55 | 0.13 | -0.91 | 0.01 |
| 30S (SSU) ribosome | 30S ribosomal protein S4 | RpsD | -0.76 | 0.01 | 0.35 | 0.00 | -0.08 | 0.63 | -0.37 | 0.00 |
| 30S (SSU) ribosome | 30S ribosomal protein S18 | RspR | -1.44 | 0.00 | 0.62 | 0.01 | 0.05 | 0.61 | -0.54 | 0.00 |
| 50S (LSU) ribosome | 50S ribosomal protein L24 | RpsX | -0.46 | 0.03 | 0.15 | 0.03 | 0.16 | 0.26 | -0.54 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| early peptidoglycan biosynthesis | UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2 | MurA2 | -2.25 | 0.00 | -1.93 | 0.04 | 0.53 | 0.43 | 0.81 | 0.01 |
| fermentation; TCA cycle | malate dehydrogenase (malic enzyme) | MleA | -3.05 | 0.00 | 3.42 | 0.00 | 0.73 | 0.37 | 0.67 | 0.02 |
| glycolysis | fructose-bisphosphate aldolase | Fba2 | -0.63 | 0.00 | -1.50 | 0.00 | -0.10 | 0.81 | 0.47 | 0.02 |
| peptidase for amino acid acquistion | Xaa-Pro dipeptidyl-peptidase | PepX | 1.51 | 0.00 | 2.15 | 0.04 | 0.73 | 0.37 | -3.14 | 0.00 |
| peptidase for amino acid acquistion | dipeptidase B | PepDB | 0.94 | 0.00 | 0.65 | 0.05 | -0.64 | 0.07 | -0.65 | 0.02 |
| polyamine uptake? | spermidine/putrescine import ATP-binding protein PotA | PotA | -0.77 | 0.03 | 1.53 | 0.00 | 0.29 | 0.42 | 1.58 | 0.00 |
| protein synthesis; ribosome function | GTP-binding protein TypA/BipA | TypA/BipA | -0.60 | 0.01 | 1.02 | 0.00 | -0.27 | 0.09 | 1.65 | 0.00 |
| purine biosynthesis | adenylosuccinate lyase | PurB | 0.94 | 0.01 | -1.17 | 0.00 | 0.21 | 0.23 | 1.35 | 0.00 |
| recycling defective proteins | ATP-dependent Clp protease ATP-binding subunit ClpE | ClpE | 1.33 | 0.02 | 2.03 | 0.00 | -0.25 | 0.26 | 1.27 | 0.00 |
| regulation of purine biosynthesis genes | Pur operon repressor | PurR2 | 1.43 | 0.01 | 0.63 | 0.02 | 0.32 | 0.13 | 2.44 | 0.00 |
| teichoic acid decoration | UDP-glucose 4-epimerase | GalE | -0.52 | 0.03 | -1.00 | 0.03 | 0.26 | 0.25 | 1.11 | 0.00 |
| 50S (LSU) ribosome | 50S ribosomal protein L10 | RplJ | -0.88 | 0.00 | -0.51 | 0.01 | -0.13 | 0.63 | -0.12 | 0.26 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 50S (LSU) ribosome | 50S ribosomal protein L6 | RplF | -1.08 | 0.00 | 1.57 | 0.00 | 0.05 | 0.70 | 0.07 | 0.67 |
| 50S (LSU) ribosome | 50S ribosomal protein L30 | RpmD | -1.24 | 0.02 | 0.67 | 0.01 | 0.42 | 0.38 | -0.39 | 0.07 |
| 50S (LSU) ribosome | 50S ribosomal protein L18 | RplR | -1.45 | 0.00 | -0.45 | 0.02 | -0.42 | 0.59 | -0.43 | 0.50 |
| fatty acid biosynthesis | acyl carrier protein | AcpP2 | 1.54 | 0.00 | 0.90 | 0.01 | 0.56 | 0.16 | 0.36 | 0.14 |
| Glycolysis/gluconeogenesis | phosphoenolpyruvate carboxykinase | | -2.04 | 0.01 | -1.63 | 0.01 | 1.51 | 0.08 | 0.74 | 0.37 |
| GMP biosynthesis | xanthine phosphoribosyltransferase | Xpt | 0.61 | 0.01 | -2.58 | 0.01 | -0.33 | 0.25 | 0.84 | 0.14 |
| late peptidoglycan biosynthesis | D-alanine--D-alanine ligase | Ddl | -1.32 | 0.00 | 0.56 | 0.01 | 0.28 | 0.37 | 0.14 | 0.57 |
| phosphonate uptake | phosphonate ABC transporter substrate-binding protein | PhnD | -0.83 | 0.02 | -2.35 | 0.00 | 0.07 | 0.81 | -0.01 | 0.99 |
| protein disaggregation (during rapid growth) | alpha-crystallin domain heat shock protein | | -4.77 | 0.00 | -3.53 | 0.00 | 1.21 | 0.37 | | . |
| protein disaggregation (during rapid growth) | alpha-crystallin domain heat shock protein | Hsp18 | -6.12 | 0.00 | -5.90 | 0.00 | | . | | . |
| protein synthesis; translation elongation | elongation factor Tu | Tuf | -0.27 | 0.01 | 0.62 | 0.00 | -0.25 | 0.07 | 0.01 | 0.70 |
| protein synthesis; translation elongation | elongation factor Ts | Tsf | -0.73 | 0.00 | -0.43 | 0.02 | 0.63 | 0.05 | 0.12 | 0.21 |

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|---|---|---|---|---|---|---|---|---|---|---|
| purine biosynthesis | phosphoribosylamine--glycine ligase | PurD | 1.34 | 0.00 | -2.38 | 0.04 | 0.37 | 0.23 | -0.02 | 0.95 |
| recycling defective proteins | ATP-dependent Clp protease ATP-binding subunit | ClpC | -4.98 | 0.00 | -2.99 | 0.00 | 1.17 | 0.17 | 0.87 | 0.37 |
| regulation of phosphate uptake genes | phosphate transport system regulatory protein phoU | PhoU | -1.58 | 0.00 | -1.97 | 0.01 | -0.97 | 0.34 | -0.49 | 0.52 |
| transcription elongation | transcription elongation protein NusA | NusA | -0.81 | 0.00 | -1.15 | 0.01 | 0.01 | 0.93 | 0.31 | 0.14 |
| 30S (SSU) ribosome | 30S ribosomal protein S3 | RpsC | -0.93 | 0.02 | 0.03 | 0.78 | 0.52 | 0.02 | -0.26 | 0.00 |
| 30S (SSU) ribosome | 30S ribosomal protein S10 | RpsJ | -1.41 | 0.00 | 0.00 | 0.98 | 1.27 | 0.00 | -0.60 | 0.03 |
| 50S (LSU) ribosome | 50S ribosomal protein L17 | RpsQ | -0.68 | 0.02 | 0.69 | 0.11 | 0.48 | 0.04 | -2.04 | 0.01 |
| 50S (LSU) ribosome | 50S ribosomal protein L23 | RplW | -0.79 | 0.00 | 0.36 | 0.13 | -0.42 | 0.04 | -0.21 | 0.03 |
| 50S (LSU) ribosome | 50S ribosomal protein L20 | RplT | -1.00 | 0.01 | -0.18 | 0.76 | 0.68 | 0.04 | -0.87 | 0.01 |
| cell division; cell shape directing complex | cell-shape determining protein Mbl | Mbl | -1.01 | 0.00 | 0.04 | 0.84 | 2.64 | 0.00 | 1.48 | 0.00 |
| deoxynucleotide biosynthesis for DNA | ribonucleoside-diphosphate reductase subunit alpha 2 | NrdE2 | -2.69 | 0.00 | -0.34 | 0.58 | 1.51 | 0.02 | 1.63 | 0.00 |
| DNA topology change | DNA-binding protein HU | Hup/Hbs | 0.52 | 0.00 | 0.09 | 0.25 | 0.29 | 0.02 | 0.48 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| fatty acid biosynthesis | 3-oxoacyl-ACP synthase I/II | FabF | 4.42 | 0.00 | | . | 4.73 | 0.00 | 1.24 | 0.01 |
| fatty acid biosynthesis | acetyl-CoA carboxylase biotin carboxylase subunit | AccC | 2.23 | 0.03 | | . | 2.13 | 0.00 | 2.76 | 0.00 |
| fatty acid biosynthesis | 3-oxoacyl-ACP synthase III | FabH | 1.88 | 0.03 | | . | 2.63 | 0.00 | 2.20 | 0.00 |
| galactose/tagatose catabolism | galactose 1-epimerase, aldos_epim superfamily | GalM | 1.88 | 0.01 | | . | 1.58 | 0.01 | 1.62 | 0.00 |
| guanosine/guanine/GMP biosynthesis | inosine-5'-monophosphate dehydrogenase | GuaB | 0.79 | 0.05 | -0.52 | 0.49 | -0.90 | 0.00 | 3.95 | 0.00 |
| L-cysteine biosynthesis | cysteine synthase | CysK | 2.63 | 0.00 | 0.32 | 0.69 | -0.98 | 0.00 | 2.95 | 0.00 |
| membrane protein assemby/recycling | ATP-dependent zinc metalloprotease FtsH | FtsH | -1.05 | 0.01 | 0.35 | 0.16 | -1.11 | 0.01 | 2.61 | 0.00 |
| methylglyoxal removal via acetol | 2,5-diketo-D-gluconic acid reductase B | DkgB | -0.49 | 0.03 | -0.55 | 0.12 | -1.19 | 0.00 | 0.85 | 0.00 |
| mevalonate pathway for isoprenoid backbones | hydroxymethylglutaryl-CoA synthase | MvaS | -1.67 | 0.01 | -1.13 | 0.17 | 2.45 | 0.00 | -0.82 | 0.03 |
| N-acetylglucosamine catabolism | N-acetylglucosamine-6-phosphate deacetylase | NagA | 0.48 | 0.03 | 0.02 | 0.86 | -0.77 | 0.02 | -0.53 | 0.00 |
| oligopeptide uptake | oligopeptide ABC-type transporter, ATP-binding protein | OppF2 | 2.84 | 0.02 | | . | 1.49 | 0.01 | 3.32 | 0.00 |
| oligopeptide uptake | oligopeptide ABC-type transporter, substrate-binding protein | OppA2 | -0.53 | 0.02 | | . | 1.03 | 0.00 | 5.49 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| peptidase for amino acid acquistion | aminopeptidase C | PepC2 | -0.48 | 0.05 | 0.56 | 0.10 | 1.27 | 0.00 | -0.28 | 0.00 |
| protein synthesis; tRNA charging | threonine--tRNA ligase | ThrS | 0.84 | 0.00 | -0.04 | 0.86 | -0.59 | 0.00 | 0.40 | 0.01 |
| protein synthesis; tRNA charging | glutamyl-tRNA(Gln) amidotransferase subunit A | GatA | -0.72 | 0.00 | 0.00 | 0.99 | 0.52 | 0.01 | 0.57 | 0.03 |
| purine biosynthesis | phosphoribosylformylglycinamidine synthase | PurS | 2.57 | 0.00 | | . | -0.45 | 0.02 | 3.10 | 0.00 |
| recycling defective proteins | chaperone protein ClpB | ClpB | 0.60 | 0.03 | -0.37 | 0.54 | -2.19 | 0.00 | 2.98 | 0.00 |
| ribosome-associated (stabilisation); attenuates translation | ribosome-associated sigma 54 modulation protein | Hpf | -0.78 | 0.00 | -0.06 | 0.88 | 1.31 | 0.00 | 2.22 | 0.00 |
| teichoic acid D-alanylation | D-alanine-poly(phosphoribitol) ligase | DltA | 1.19 | 0.01 | 0.01 | 0.94 | -1.58 | 0.00 | -0.29 | 0.03 |
| thiol group reduction (similar to glutathione) | coenzyme A disulfide reductase | | -2.43 | 0.00 | -0.97 | 0.25 | 3.52 | 0.00 | -1.93 | 0.02 |
| 50S (LSU) ribosome | 50S ribosomal protein L27 | RpmA | -0.73 | 0.03 | 0.21 | 0.14 | 1.24 | 0.03 | -0.95 | 0.12 |
| 50S (LSU) ribosome | 50S ribosomal protein L9 | RplI | -1.46 | 0.00 | 0.18 | 0.51 | 1.11 | 0.00 | -0.48 | 0.13 |
| early peptidoglycan biosynthesis | glucosamine--fructose-6-phosphate aminotransferase | GlmS | -1.46 | 0.00 | 0.17 | 0.27 | 3.66 | 0.00 | -0.01 | 0.95 |
| fatty acid biosynthesis | malonyl CoA-acyl carrier protein transacylase | FabD | 2.71 | 0.04 | 0.72 | 0.37 | 0.94 | 0.02 | 1.24 | 0.12 |

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|---|---|---|---|---|---|---|---|---|---|---|
| fatty acid biosynthesis | 3-oxoacyl-ACP reductase | FabG | 2.41 | 0.00 | | . | 1.69 | 0.02 | 0.87 | 0.11 |
| fatty acid degradation or detoxification? | fatty acid hydratase/isomerase (oleate hydratase, linoleate isomerase) | Sph | 2.41 | 0.00 | | . | -1.69 | 0.00 | 0.43 | 0.05 |
| fermentation; end-product metabolism | pyruvate oxidase | Pox5 | -2.30 | 0.00 | -0.75 | 0.37 | 2.24 | 0.00 | 1.90 | 0.12 |
| fructose catabolism | 1-phosphofructokinase | FruK | -3.69 | 0.00 | -1.24 | 0.12 | 0.99 | 0.03 | 1.06 | 0.13 |
| L-arginine biosynthesis; pyrimidine biosynthesis | carbamoyl-phosphate synthase large subunit | CarB | 2.98 | 0.00 | -0.52 | 0.49 | 3.15 | 0.00 | 1.24 | 0.12 |
| metabolism of aspartate | aspartate racemase | | -0.27 | 0.04 | -0.89 | 0.18 | 2.56 | 0.00 | -0.55 | 0.11 |
| one carbon pool by folate | formate--tetrahydrofolate ligase | Fhs | 1.27 | 0.02 | -1.34 | 0.08 | -0.40 | 0.03 | 0.22 | 0.39 |
| oxidative stress management | Dyp-type iron-dependent peroxidase | YfeX | -2.68 | 0.01 | -0.32 | 0.27 | 0.70 | 0.00 | 0.80 | 0.05 |
| peptidoglycan turnover | muramoyl-tetrapeptide L,D-carboxypeptidase | | 1.79 | 0.02 | -1.17 | 0.34 | 1.35 | 0.02 | 0.14 | 0.64 |
| phospholipid biosynthesis | glycerol-3-phosphate dehydrogenase | GpsA | -1.20 | 0.01 | -1.15 | 0.11 | 1.65 | 0.02 | 1.17 | 0.10 |
| protein phosphorylation (regulation)> | tyrosine-protein phosphatase | Ptp | -1.10 | 0.02 | -0.59 | 0.51 | 1.85 | 0.02 | 0.74 | 0.17 |
| protein secretion; SRP pathway (trafficking) | signal recognition particle protein | Ffh | -1.30 | 0.00 | 1.02 | 0.15 | 1.02 | 0.01 | 0.60 | 0.27 |

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|---|---|---|---|---|---|---|---|---|---|---|
| protein synthesis; N-formylMet initiator removal | peptide deformylase | Def | 0.67 | 0.02 | 0.40 | 0.15 | 0.82 | 0.04 | -0.03 | 0.91 |
| protein synthesis; tRNA charging | isoleucine--tRNA ligase | IleS | -2.15 | 0.00 | -0.28 | 0.23 | 0.82 | 0.01 | -0.50 | 0.09 |
| purine biosynthesis | phosphoribosylformylglycinamidine cyclo-ligase | PurM | 1.34 | 0.05 | 0.45 | 0.55 | 1.85 | 0.02 | -1.27 | 0.12 |
| quorum sensing?; detoxification? | S-ribosylhomocysteine lyase | LuxS | 3.25 | 0.02 | -0.34 | 0.31 | -4.52 | 0.02 | | . |
| resistance to cell envelop targeted toxic substances such as nisin | TelA superfamily protein | YaaN | 1.30 | 0.01 | 0.39 | 0.43 | -1.73 | 0.03 | -1.23 | 0.37 |
| ribosomal RNA processing (LSU) | 23S rRNA methyltransferase, putative | YefA | -1.47 | 0.01 | 1.53 | 0.07 | 2.55 | 0.05 | 0.52 | 0.50 |
| ribosome processing (LSU); RNA chaperone | DEAD/DEAH box helicase | CshB | -2.99 | 0.00 | -0.70 | 0.20 | -1.85 | 0.00 | -1.62 | 0.18 |
| RNA polymerase | DNA-directed RNA polymerase subunit beta | RpoB | -1.23 | 0.00 | -0.33 | 0.15 | 1.41 | 0.00 | 0.10 | 0.37 |
| teichoic acid decoration | dTDP-4-dehydrorhamnose 3,5-epimerase | | 5.15 | 0.04 | | . | -0.94 | 0.02 | 0.28 | 0.28 |
| tRNA modification | tRNA-specific 2-thiouridylase | MnmA | -3.78 | 0.01 | | . | 3.94 | 0.01 | 0.21 | 0.60 |
| 30S (SSU) ribosome | 30S ribosomal protein S21 | RpsU | -0.96 | 0.02 | 0.19 | 0.50 | 1.02 | 0.10 | -1.78 | 0.00 |
| 30S (SSU) ribosome | 30S ribosomal protein S13 | RpsM | -1.05 | 0.01 | -0.23 | 0.37 | 0.43 | 0.10 | -0.88 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 30S (SSU) ribosome | 30S ribosomal protein S11 | RpsK | -1.20 | 0.01 | 0.14 | 0.26 | 0.00 | 1.00 | -0.71 | 0.00 |
| 50S (LSU) ribosome | 50S ribosomal protein L3 | RplC | -0.97 | 0.00 | 0.14 | 0.18 | 0.06 | 0.88 | -1.00 | 0.01 |
| 50S (LSU) ribosome | 50S ribosomal protein L13 | RplM | -1.06 | 0.05 | -0.42 | 0.12 | -0.14 | 0.52 | -0.54 | 0.00 |
| amino acid transmamination? | transcriptional regulator (WHTH_GntR superfamily) fused with a PLP dep. | | 5.10 | 0.00 | | . | -0.79 | 0.37 | 2.70 | 0.04 |
| cell wall alteration signalling | cell wall integrity sensing response regulator CesR | CesR | -1.18 | 0.00 | -0.32 | 0.57 | -0.69 | 0.33 | 1.29 | 0.01 |
| DNA repair (homologous recombination; SOS response) | ATP-dependent helicase/nuclease subunit A | AddA | 2.57 | 0.02 | -0.75 | 0.37 | 0.51 | 0.36 | 1.62 | 0.00 |
| early peptidoglycan biosynthesis | phosphoglucosamine mutase | GlmM | -1.05 | 0.01 | -0.25 | 0.06 | -0.08 | 0.67 | -0.39 | 0.02 |
| fatty acid biosynthesis | enoyl-[acyl-carrier protein] reductase II | FabK | 2.80 | 0.00 | | . | 0.20 | 0.28 | 0.69 | 0.00 |
| fatty acid biosynthesis | (3R)-hydroxymyristoyl-ACP dehydratase | FabZ | 2.23 | 0.00 | | . | -0.32 | 0.41 | 2.57 | 0.00 |
| fermentation, end-product formation | phosphate acetyltransferase | Pta | -0.19 | 0.01 | -0.18 | 0.10 | -0.11 | 0.66 | 0.74 | 0.01 |
| fructose uptake | PTS (fructose family) subunit IIABC | FruA3 | -2.43 | 0.00 | -0.89 | 0.10 | 1.06 | 0.13 | 3.88 | 0.00 |
| glycolysis | pyruvate dehydrogenase E1 component subunit beta | PdhB | 0.65 | 0.04 | 0.07 | 0.89 | 0.80 | 0.10 | 1.91 | 0.02 |

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|---|---|---|---|---|---|---|---|---|---|---|
| glycolysis | pyruvate kinase | Pyk | 0.53 | 0.01 | 0.16 | 0.14 | 0.01 | 0.95 | 0.05 | 0.04 |
| glycolysis | glucokinase | GlcK | -2.46 | 0.00 | -0.23 | 0.27 | -0.24 | 0.57 | -2.62 | 0.00 |
| mannitol catabolism | mannitol-1-phosphate 5-dehydrogenase | MtlD3 | -0.28 | 0.01 | 0.17 | 0.34 | -0.22 | 0.32 | -1.94 | 0.00 |
| oligopeptide uptake | oligopeptide ABC-type transporter ATP-binding protein | OppD2 | 1.54 | 0.01 | | . | 1.30 | 0.06 | 3.11 | 0.00 |
| peptidase for amino acid acquistion | oligoendopeptidase F | YjbG2 | 2.71 | 0.01 | | . | 0.28 | 0.46 | 1.11 | 0.04 |
| protein synthesis; tRNA charging | Valine--tRNA ligase | ValS | -0.22 | 0.02 | -0.59 | 0.10 | -0.05 | 0.80 | 1.36 | 0.00 |
| protein synthesis; tRNA charging | arginine--tRNA ligase | ArgS | -1.10 | 0.00 | 0.21 | 0.35 | 0.92 | 0.06 | 1.95 | 0.00 |
| purine biosynthesis | phosphoribosylaminoimidazole carboxamide formyltransferase/inosine-monophosphate | PurH | 4.10 | 0.00 | | . | 0.04 | 0.67 | 3.21 | 0.03 |
| purine nucleoside interconversion/salvage | GMP reductase | GuaC | 1.81 | 0.00 | -1.18 | 0.09 | 0.00 | 0.99 | 1.05 | 0.02 |
| ribosomal RNA processing | ribonuclease R | Rnr | -2.24 | 0.00 | -0.54 | 0.06 | 0.53 | 0.25 | -1.10 | 0.00 |
| S-adenosylmethionine biosynthesis | S-adenosylmethionine synthase | MetK | 0.99 | 0.04 | 0.33 | 0.30 | -0.45 | 0.12 | 0.46 | 0.02 |
| succinate/GABA/aldehyde metabolism? | succinate-semialdehyde dehydrogenase [NADP(+)] | GabD | 1.23 | 0.01 | -0.75 | 0.37 | -0.38 | 0.12 | 1.17 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| trehalose catabolism | trehalose-6-phosphate hydrolase | TreA | -2.26 | 0.00 | | . | | . | 1.80 | 0.01 |
| uracil/uridine/UMP/UTP biosynthesis | uridylate kinase | PyrH | -1.71 | 0.00 | 0.86 | 0.14 | 0.32 | 0.52 | 1.61 | 0.01 |
| 30S (SSU) ribosome | 30S ribosomal protein S7 | RpsG | -0.36 | 0.04 | -0.40 | 0.08 | -0.03 | 0.67 | -0.33 | 0.09 |
| 30S (SSU) ribosome | 30S ribosomal protein S2 | RpsB | -1.11 | 0.00 | 0.12 | 0.53 | -0.38 | 0.12 | -0.06 | 0.41 |
| 50S (LSU) ribosome | 50S ribosomal protein L7/L12 | RplL | -0.59 | 0.01 | -0.04 | 0.26 | 0.14 | 0.29 | 0.21 | 0.30 |
| 50S (LSU) ribosome | 50S ribosomal protein L5 | RplE | -0.76 | 0.00 | 0.09 | 0.52 | 0.03 | 0.74 | -0.09 | 0.57 |
| 50S (LSU) ribosome | 50S ribosomal protein L29 | RpmC | -1.00 | 0.01 | 0.36 | 0.23 | 0.26 | 0.45 | 0.02 | 0.70 |
| 50S (LSU) ribosome | 50S ribosomal protein L14 | RplN | -1.00 | 0.02 | -0.24 | 0.10 | 0.56 | 0.09 | 0.37 | 0.08 |
| 50S (LSU) ribosome | 50S ribosomal protein L15 | RplO | -1.17 | 0.00 | -0.25 | 0.08 | 0.03 | 0.89 | -0.08 | 0.19 |
| 50S (LSU) ribosome | 50S ribosomal protein L4 | RplD | -1.72 | 0.00 | -0.01 | 0.90 | 0.04 | 0.83 | 0.38 | 0.05 |
| 50S (LSU) ribosome | 50S ribosomal protein L19 | RplS | -2.13 | 0.01 | 0.37 | 0.07 | 0.06 | 0.61 | -0.20 | 0.10 |
| adhesion | fibrinogen/fibronectin(RNA)-binding protein FpbA | YloA | -1.37 | 0.05 | 0.72 | 0.37 | 1.20 | 0.12 | | . |

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|---|---|---|---|---|---|---|---|---|---|---|
| ADP biosynthesis; energy homeostasis | adenylate kinase | Adk | -0.82 | 0.05 | -0.13 | 0.38 | -0.20 | 0.21 | 0.21 | 0.25 |
| ATP synthesis, H+ extrusion | ATP synthase subunit delta | AtpH | 1.99 | 0.00 | 0.68 | 0.40 | 1.21 | 0.12 | 0.77 | 0.37 |
| beta-glucoside uptake | PTS system beta-glucoside-specific transporter subunit IIBCA | BglP | -2.27 | 0.03 | | . | 1.57 | 0.16 | -0.74 | 0.37 |
| calcium uptake | calcium-translocating P-type ATPase | | 1.51 | 0.00 | -0.03 | 0.97 | -1.31 | 0.12 | 0.00 | 1.00 |
| cell division; divisome complex | cell division ATP-binding protein FtsE | FtsE | -2.22 | 0.00 | -0.72 | 0.16 | -0.42 | 0.50 | 2.71 | 0.09 |
| cell division; divisome complex | cell division protein FtsX | FtsX | -2.60 | 0.00 | -1.24 | 0.37 | -0.79 | 0.37 | 0.74 | 0.37 |
| conjugation? | sex pheromone lipoprotein (similar to cAD1 in E. faecalis) | | -1.67 | 0.00 | -0.75 | 0.17 | 1.21 | 0.12 | -0.74 | 0.37 |
| conjugation-associated? | sex pheromone lipoprotein, CamS superfamily | YerH | -1.53 | 0.00 | -0.75 | 0.37 | -0.79 | 0.37 | 0.74 | 0.37 |
| DNA repair (Uvr excision repair complex; SOS response) | excinuclease ATPase subunit A | UvrA | -1.83 | 0.02 | -0.75 | 0.37 | -0.66 | 0.27 | -0.25 | 0.74 |
| fatty acid biosynthesis | acetyl-CoA carboxylase biotin carboxyl carrier protein | AccB | 2.84 | 0.02 | | . | -0.15 | 0.54 | -0.02 | 0.94 |
| fatty acid biosynthesis | 3-oxoacyl-[acyl-carrier-protein] reductase 4 | Bkr4 | 2.67 | 0.01 | | . | 0.02 | 0.93 | 0.73 | 0.17 |
| fermentation; end-product formation | L-lactate dehydrogenase | Ldh | 0.42 | 0.00 | -0.14 | 0.17 | 0.30 | 0.10 | -0.03 | 0.68 |

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|---|---|---|---|---|---|---|---|---|---|---|
| Fe-S cluster assembly | Fe-S cluster assembly, ATP-binding protein | SufC | 0.94 | 0.01 | -0.70 | 0.20 | 0.08 | 0.51 | 0.26 | 0.56 |
| glycine betaine uptake | glycine betaine transport system permease protein | OpuAB | -1.53 | 0.00 | 0.72 | 0.37 | -0.81 | 0.37 | | . |
| glycine betaine uptake | Glycine betaine transport ATP-binding protein | OpuAA | -2.59 | 0.01 | | . | 0.40 | 0.61 | 1.26 | 0.37 |
| glycolysis | dihydrolipoyl dehydrogenase | PdhD | 2.17 | 0.00 | -0.27 | 0.25 | 0.81 | 0.15 | -0.68 | 0.22 |
| glycolysis | triosephosphate isomerase | Tpi | 0.22 | 0.02 | -0.08 | 0.58 | -0.06 | 0.45 | 0.12 | 0.12 |
| H2O2/hydroperoxide removal | glutathione peroxidase | Gpo | 2.84 | 0.01 | 0.80 | 0.14 | -0.76 | 0.25 | 0.20 | 0.66 |
| L-arginine biosynthesis | argininosuccinate synthase | ArgG | -1.83 | 0.02 | -0.75 | 0.37 | -1.30 | 0.12 | | . |
| L-aspartate biosynthesis | L-asparaginase | AnsA | -2.06 | 0.01 | | . | 0.26 | 0.55 | 0.28 | 0.41 |
| late peptidoglycan biosynthesis | D-alanyl-D-alanine carboxypeptidase dacA | DacA | -3.22 | 0.00 | 0.42 | 0.20 | -0.76 | 0.10 | 0.52 | 0.09 |
| L-glutamate biosynthesis | glutamate synthase [NADPH] large subunit | GltA | -1.83 | 0.02 | 0.72 | 0.37 | 0.26 | 0.55 | -0.03 | 0.96 |
| L-methionine biosynthesis | 5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase | MetE | -0.68 | 0.05 | -1.24 | 0.12 | -0.42 | 0.54 | 0.62 | 0.08 |
| L-methionine biosynthesis | cystathionine beta-lyase PatB | PatB2 | -2.23 | 0.00 | -0.05 | 0.89 | -0.08 | 0.61 | 1.20 | 0.07 |

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|---|---|---|---|---|---|---|---|---|---|---|
| L-methionine salvage; quorum sensing?; detoxification? | 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase | Mtn | -3.30 | 0.01 | -0.70 | 0.40 | 0.96 | 0.23 | 0.67 | 0.21 |
| L-proline biosynthesis | pyrroline-5-carboxylate reductase | ProC | 2.41 | 0.03 | -1.24 | 0.37 | -0.34 | 0.16 | 0.01 | 0.99 |
| L-threonine metabolism; Isoleucine biosynthesis | L-threonine dehydratase catabolic TdcB | TdcB | -2.06 | 0.01 | -0.75 | 0.37 | -0.08 | 0.93 | 0.02 | 0.98 |
| maltose/maltodextrin uptake | maltose/maltodextrin ABC-type transporter, permease component | MdxF/MalF | 1.51 | 0.00 | -1.24 | 0.12 | | . | | . |
| N-acetylglucosamine catabolism | N-acetylglucosamine-6-phosphate deacetylase | ManD | -1.53 | 0.00 | | . | 0.12 | 0.83 | -0.05 | 0.94 |
| nucleoside salvage; phosphate metabolism | 5'-nucleotidase/UDP-sugar hydrolase, MPP_UshA_like family | YunD | -1.53 | 0.00 | 1.20 | 0.12 | -0.79 | 0.37 | -0.03 | 0.97 |
| pantothenate/CoA biosynthesis | Pantothenate kinase | CoaA | 1.51 | 0.00 | 0.72 | 0.37 | | . | | . |
| peptide relase factor modification | release factor glutamine methyltransferase | PrmC | 1.79 | 0.02 | 1.56 | 0.16 | | . | | . |
| polar amino acid uptake | polar amino acid ABC-type exporter, permease component | GlnP | -1.83 | 0.02 | -0.89 | 0.36 | -0.79 | 0.37 | | . |
| porphyrin ring biosynthesis | ferrochelatase | HemH | 2.23 | 0.03 | -1.24 | 0.12 | 1.30 | 0.17 | -0.02 | 0.98 |
| protein secretion: exported protein folding | foldase protein prsA | PrsA | -1.09 | 0.00 | 0.12 | 0.67 | 0.73 | 0.37 | 0.54 | 0.09 |
| protein secretion: Sec system (insertion) | preprotein translocase insertion/stabilisation protein | YajC | -0.98 | 0.00 | -0.06 | 0.86 | | . | 1.60 | 0.15 |

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|---|---|---|---|---|---|---|---|---|---|---|
| protein synthesis; translation elongation | elongation factor P | Efp | -0.74 | 0.02 | -0.32 | 0.60 | -0.24 | 0.20 | 0.29 | 0.19 |
| protein synthesis; translation elongation | elongation factor G | Fus | -1.15 | 0.00 | 0.16 | 0.06 | 0.20 | 0.06 | 0.06 | 0.45 |
| protein synthesis; translation initiation | translation initiation factor IF-3 | InfC | -1.05 | 0.00 | -0.65 | 0.06 | 0.62 | 0.06 | -0.16 | 0.39 |
| protein synthesis; tRNA charging | Proline--tRNA ligase | ProS | -1.06 | 0.02 | -0.49 | 0.22 | 0.15 | 0.50 | 0.18 | 0.17 |
| purine biosynthesis | Phosphoribosylaminoimidazole-succinocarboxamide synthase | PurC | 4.65 | 0.00 | | . | -0.20 | 0.46 | 1.61 | 0.09 |
| pyrimidine nucleoside salvage | uracil phosphoribosyltransferase | Upp | -0.70 | 0.00 | -0.36 | 0.09 | 0.15 | 0.55 | 0.43 | 0.16 |
| recycling defective proteins | ATP-dependent Clp protease ATP-binding subunit ClpX | ClpX | -1.99 | 0.00 | -0.07 | 0.73 | -0.23 | 0.18 | 0.21 | 0.05 |
| regulatory role unknown | GntR family transcriptional regulator | | 1.51 | 0.00 | 0.72 | 0.37 | 0.78 | 0.41 | -0.90 | 0.36 |
| riboflavin/FMN synthesis? | riboflavin biosynthesisn acetyltransferase | RibT | 2.23 | 0.03 | -0.75 | 0.37 | | . | -0.77 | 0.37 |
| ribosomal RNA processing (LSU) | 23S rRNA methyltransferase, SpoU_sub_bind/SpoU_methylase superfamily | YsgA | -1.58 | 0.00 | 0.45 | 0.55 | 0.26 | 0.74 | -0.01 | 0.56 |
| RNA degradation (RNA degradosome) | ribonuclease J1 | RnjA | -1.36 | 0.01 | -0.68 | 0.38 | 0.17 | 0.79 | 0.00 | 1.00 |
| RNA polymerase | DNA-directed RNA polymerase subunit omega | RpoZ | 1.26 | 0.02 | -0.24 | 0.26 | -0.29 | 0.55 | -0.01 | 0.98 |

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|---|---|---|---|---|---|---|---|---|---|---|
| sodium homeostasis | Na+ efflux ATP-typetransporter, ATP-binding component | NatA | -1.53 | 0.00 | 0.72 | 0.37 | -0.81 | 0.37 | 0.74 | 0.37 |
| teichoic acid biosynthesis | polyprenyl glycosylphosphotransferase | | -1.53 | 0.00 | 0.72 | 0.37 | | . | 0.02 | 0.98 |
| teichoic acid biosynthesis | monoglucosyldiacylglycerol glycosyltransferase | | -2.16 | 0.01 | -1.24 | 0.37 | | . | | . |
| teichoic acid D-alanylation | D-alanine-poly(phosphoribitol) ligase subunit 2 | DltC | 2.03 | 0.01 | | . | | . | | . |
| tRNA modification | tRNA (guanine-N(7)-)-methyltransferase | TrmB | -1.81 | 0.01 | | . | 0.56 | 0.51 | 0.85 | 0.38 |
| tRNA modification | tRNA-dihydrouridine synthase 1 | Dus1 | -2.42 | 0.02 | -0.23 | 0.70 | 1.23 | 0.18 | 0.66 | 0.07 |
| UMP/uridine/uracil biosynthesis | aspartate carbamoyltransferase | PyrB | 3.74 | 0.00 | 1.20 | 0.37 | -0.93 | 0.16 | 1.62 | 0.16 |
| UMP/uridine/uracil biosynthesis | dihydroorotase | PyrC | 2.23 | 0.03 | -0.75 | 0.37 | -0.54 | 0.09 | 0.77 | 0.37 |
| UMP/uridine/uracil biosynthesis | dihydroorotate dehydrogenase A (fumarate) | PyrD | 1.51 | 0.00 | -0.75 | 0.37 | 0.62 | 0.43 | 1.63 | 0.16 |
| UMP/uridine/uracil biosynthesis | hypoxanthine-guanine phosphoribosyltransferase | PyrR/Hpt | -1.27 | 0.02 | -0.32 | 0.07 | 0.04 | 0.93 | 0.47 | 0.17 |
| glucose/mannose uptake | PTS (Mannose/Fructose/Sorbose family) IID | ManZ | -0.02 | 0.96 | 1.84 | 0.02 | -2.78 | 0.00 | 1.80 | 0.01 |
| glycolysis | phosphoglucomutase | PgcA | 0.37 | 0.19 | -0.74 | 0.01 | 0.45 | 0.00 | 0.42 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| glycolysis | 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase | GpmA2 | -0.15 | 0.39 | 0.55 | 0.00 | -0.63 | 0.04 | -0.14 | 0.00 |
| mevalonate pathway for isoprenoid backbones | mevalonate kinase | MvaK1 | -0.20 | 0.73 | 2.12 | 0.02 | -0.95 | 0.03 | -1.09 | 0.02 |
| N-acetylglucosamine catabolism | glucosamine-6-phosphate deaminase | NagB | 0.03 | 0.83 | -0.78 | 0.04 | -0.85 | 0.02 | 0.70 | 0.00 |
| nascent protein folding | trigger factor Tig | Tig | 0.06 | 0.56 | 1.06 | 0.00 | 0.73 | 0.03 | 0.13 | 0.00 |
| nascent protein folding | class I heat-shock protein (chaperonin) small subunit | GroES | -0.30 | 0.18 | -0.83 | 0.01 | 1.33 | 0.02 | -1.61 | 0.00 |
| peptidase for amino acid acquistion | tripeptide aminopeptidase T | PepT | 2.23 | 0.12 | 4.25 | 0.00 | -2.76 | 0.02 | -3.65 | 0.00 |
| peptidase for amino acid acquistion | beta-Ala-Xaa dipeptidase | PepV | -0.31 | 0.13 | -1.13 | 0.01 | 0.35 | 0.04 | 1.67 | 0.00 |
| peptidase for amino acid acquistion | dipeptidase, peptidase C69 family | PepD | -0.81 | 0.19 | -0.99 | 0.01 | 2.87 | 0.00 | 0.51 | 0.00 |
| peptidase for amino acid acquistion | cysteinyl aminopeptidase, peptidase C1 family | PepC | -1.50 | 0.16 | 1.63 | 0.04 | 1.49 | 0.01 | -4.89 | 0.00 |
| recycling defective proteins; virulence | ATP-dependent Clp protease proteolytic subunit | ClpP2 | 0.36 | 0.07 | 0.75 | 0.00 | 1.94 | 0.00 | -1.34 | 0.00 |
| sugar/glycerol uptake | glycerol-3-phosphate ABC transporter substrate-binding protein | UgpB | -0.16 | 0.56 | -0.42 | 0.03 | 0.50 | 0.03 | 1.99 | 0.00 |
| 30S (SSU) ribosome | 30S ribosomal protein S20 | RpsT | -0.03 | 0.49 | -0.07 | 0.00 | 1.46 | 0.01 | -0.28 | 0.41 |

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|---|---|---|---|---|---|---|---|---|---|---|
| cell division; divisome complex | septum site-determining protein DivIVA | DivIVA | 0.06 | 0.76 | 0.86 | 0.03 | -0.42 | 0.03 | 0.17 | 0.22 |
| fermentation; end-product metabolism | NADH-dependent butanol dehydrogenase A | BdhA | -0.74 | 0.37 | -3.49 | 0.00 | 2.91 | 0.02 | -0.36 | 0.49 |
| glycine biosynthesis; folate biosynthesis; one carbon pool by folate | serine hydroxymethyltransferase | GlyA | 0.10 | 0.81 | -0.79 | 0.00 | 1.55 | 0.00 | 1.10 | 0.14 |
| mannose catabolism | mannose-6-phosphate isomerase | Pmi/ManA | -0.10 | 0.63 | -0.99 | 0.00 | -0.59 | 0.05 | 0.11 | 0.66 |
| pentose phosphate pathway; D-xylulose catabolism | xylulose-5-phosphate phosphoketolase | XpaK | -0.22 | 0.41 | -0.50 | 0.04 | 0.62 | 0.04 | -0.13 | 0.32 |
| peptidase for amino acid acquistion | oligoendopeptidase F | YjbG | 2.22 | 0.06 | 1.23 | 0.03 | -1.12 | 0.00 | 0.39 | 0.20 |
| protein synthesis; tRNA charging | glutamate--tRNA ligase | GltX | -0.07 | 0.62 | 0.52 | 0.05 | -1.02 | 0.00 | -0.04 | 0.73 |
| protein synthesis; tRNA charging | aspartyl/glutamyl-tRNA amidotransferase subunit B | GatB | -0.19 | 0.27 | -0.43 | 0.01 | 0.56 | 0.02 | 0.45 | 0.06 |
| recycling defective proteins | ATP-dependent protease, peptidase subunit | HslV | 0.70 | 0.45 | -1.90 | 0.02 | -1.21 | 0.00 | | . |
| 30S (SSU) ribosome | 30S ribosomal protein S19 | RpsS | -0.85 | 0.07 | 0.87 | 0.05 | -0.03 | 0.72 | -0.50 | 0.01 |
| 50S (LSU) ribosome | 50S ribosomal protein L28 | RpmB | 0.01 | 0.99 | -1.77 | 0.04 | 4.58 | 0.06 | -4.88 | 0.03 |
| amino acid uptake? | amino-acid ABC-type transporter, substrate-binding protein | | 0.69 | 0.37 | 0.27 | 0.03 | 1.21 | 0.12 | -2.72 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| ATP synthesis | manganese-dependent inorganic pyrophosphatase | PpaC | 0.36 | 0.05 | -0.89 | 0.00 | -0.19 | 0.15 | 0.38 | 0.04 |
| branched chain amino acid uptake | branched-chain amino acid ABC-type transporter, substrate binding protein | LivK | 1.51 | 0.16 | 2.00 | 0.01 | 0.40 | 0.61 | -1.99 | 0.01 |
| DNA repair (base excision) | apurinic/apyrimidinic 3'-exo-deoxyribonuclease III | exoA | -0.04 | 0.96 | -1.90 | 0.02 | 0.42 | 0.72 | -0.97 | 0.02 |
| DNA repair (base excision) | uracil-DNA glycosylase | Ung | -0.84 | 0.11 | 1.56 | 0.00 | -0.95 | 0.34 | 1.62 | 0.00 |
| exopolysaccharide biosynthesis | dTDP-glucose 4,6-dehydratase | RmlB2 | -0.97 | 0.35 | -0.91 | 0.02 | -0.23 | 0.71 | -0.29 | 0.02 |
| exopolysaccharide biosynthesis | dTDP-4-dehydrorhamnose reductase | SpsK2 | | . | -3.29 | 0.00 | | . | -4.59 | 0.01 |
| fermentation; end-product metabolism | pyruvate oxidase | YdaP | 0.26 | 0.66 | 3.99 | 0.00 | 0.72 | 0.37 | -4.67 | 0.00 |
| glycerol catabolism | glycerol dehydrogenase, GlyDH_like1 family, putative | YbdH | -0.74 | 0.37 | 2.90 | 0.00 | | . | -4.03 | 0.00 |
| lactose catabolism | 6-phospho-beta-galactosidase | LacG | | . | -1.20 | 0.00 | 0.42 | 0.58 | -4.78 | 0.00 |
| L-methionine biosynthesis | cystathionine beta-lyase | MetC | | . | -0.67 | 0.03 | | . | -2.80 | 0.00 |
| myo-inositol catabolism | myo-inositol catabolism protein IolS | IolS | 0.11 | 0.64 | -1.05 | 0.00 | -0.16 | 0.54 | 0.74 | 0.01 |
| nascent protein folding | class I heat-shock protein (chaperonin) large subunit | GroEL | -0.06 | 0.58 | -0.63 | 0.00 | -0.01 | 0.94 | -1.58 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| oligopeptide uptake | oligopeptide-binding protein OppA | OppA | 0.13 | 0.67 | 1.17 | 0.00 | 1.17 | 0.11 | -3.19 | 0.00 |
| oligopeptide uptake | di-/oligopeptide ABC transporter substrate-binding protein | DppE/OppA | -0.04 | 0.96 | 2.98 | 0.02 | | . | -1.92 | 0.02 |
| oligopeptide uptake | di-/oligopeptide ABC transporter substrate-binding protein | DppE/OppA | -0.69 | 0.37 | 2.98 | 0.00 | -0.57 | 0.64 | -1.63 | 0.00 |
| oligopeptide uptake | oligopeptide uptake ABC-type transporter, substrate-binding protein | OppA3 | -1.15 | 0.12 | 2.87 | 0.00 | -0.07 | 0.94 | -1.36 | 0.02 |
| peptidase for amino acid acquistion | glutamyl aminopeptidase, M20_dimer superfamily | YsdC | | . | 2.90 | 0.00 | | . | -4.49 | 0.00 |
| protein methionine sulfoxide reduction | peptide methionine sulfoxide reductase MsrB | MsrB | | . | 0.44 | 0.03 | | . | -3.68 | 0.00 |
| protein synthesis; N-formylMet initiator removal | methionine aminopeptidase | Map | 0.88 | 0.16 | 1.57 | 0.02 | -0.08 | 0.78 | -1.35 | 0.00 |
| protein synthesis; tRNA charging | methionine--tRNA ligase | MetS | 0.21 | 0.58 | -1.01 | 0.01 | 0.21 | 0.46 | 1.76 | 0.00 |
| protein synthesis; tRNA charging | glycine--tRNA ligase subunit beta | GlyS | -0.59 | 0.32 | 1.53 | 0.01 | -0.24 | 0.15 | -0.86 | 0.03 |
| purine biosynthesis | ribose-phosphate pyrophosphokinase 1 | Prs1 | 0.04 | 0.86 | -0.67 | 0.02 | 0.39 | 0.09 | 1.87 | 0.00 |
| stress response (phosphate starvation)? | CsbD family protein | CsbD | -0.02 | 0.49 | -1.61 | 0.00 | 0.48 | 0.29 | 1.90 | 0.01 |
| tRNA modification; iron-cluster assembly; thiamine biosynthesis | cysteine desulfurase (tRNA sulfurtransferase) | IscS | -0.40 | 0.61 | -1.61 | 0.00 | -0.76 | 0.06 | 3.10 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 50S (LSU) ribosome | 50S ribosomal protein L11 | RplK | -0.20 | 0.09 | 0.68 | 0.00 | -0.34 | 0.11 | -0.14 | 0.83 |
| cell division; septation | septum site-determining protein MinD | MinD | 0.84 | 0.11 | -0.05 | 0.00 | -0.11 | 0.91 | 0.76 | 0.37 |
| cell wall alteration signalling | cell wall integrity sensing histidine kinase CesK | CesK | -1.56 | 0.17 | -1.61 | 0.00 | | . | | . |
| detoxification | maltose O-acetyltransferase family protein | Maa | 1.16 | 0.12 | -1.15 | 0.01 | -0.27 | 0.49 | -0.02 | 0.94 |
| DNA topological change | DNA topoisomerase 4 subunit A | ParC | -0.01 | 0.99 | -1.42 | 0.04 | 0.56 | 0.51 | -0.77 | 0.16 |
| fermentation; end-product formation | acetaldehyde-CoA dehydrogenase/iron-dependent alcohol dehydrogenase/pyruvate- | AdhE | 0.69 | 0.37 | -1.15 | 0.01 | -1.31 | 0.12 | -0.77 | 0.37 |
| glycolysis | dihydroxyacetone kinase, C-terminal domain | DhaL | 0.42 | 0.18 | -0.89 | 0.02 | 0.34 | 0.33 | 0.88 | 0.12 |
| late peptidoglycan biosynthesis | UDP-N-acetylmuramoylalanine--D-glutamate ligase | MurD | 0.35 | 0.14 | 1.52 | 0.01 | -0.62 | 0.34 | -0.02 | 0.90 |
| late peptidoglycan biosynthesis | UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase | MurF | -0.37 | 0.62 | 0.68 | 0.00 | 0.09 | 0.79 | 0.81 | 0.09 |
| L-lysine biosynthesis | succinyl-diaminopimelate desuccinylase | DapE2 | 0.30 | 0.41 | -1.24 | 0.03 | -0.37 | 0.34 | 0.43 | 0.48 |
| oligopeptide uptake | oligopeptide uptake ABC-type transporter, ATP-binding protein | OppF3 | -0.74 | 0.37 | 2.29 | 0.03 | -0.96 | 0.34 | -0.47 | 0.69 |
| oligopeptide uptake | oligopeptide uptake ABC-type transporter system, permease component | OppC3 | | . | 1.56 | 0.00 | | . | 1.26 | 0.37 |

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|---|---|---|---|---|---|---|---|---|---|---|
| peptidase for amino acid acquistion | Proline iminopeptidase | FpaP3 | -0.04 | 0.85 | 1.20 | 0.00 | -0.21 | 0.23 | -0.16 | 0.11 |
| peptidase for amino acid acquistion | Xaa-Pro aminopeptidase, APP_like family | PepQ | -0.65 | 0.24 | -0.05 | 0.00 | 1.20 | 0.12 | 0.76 | 0.37 |
| protein synthesis; translation elongation | elongation factor P | Efp | -0.32 | 0.38 | -0.05 | 0.00 | 0.17 | 0.84 | 0.74 | 0.37 |
| protein synthesis; translation initiation | translation initiation factor IF-1 | InfA | -0.41 | 0.24 | 0.76 | 0.03 | -0.15 | 0.47 | 0.19 | 0.23 |
| protein synthesis; tRNA charging | purine nucleoside phosphoramidase, putative | HinT2 | 1.05 | 0.12 | -0.05 | 0.00 | -0.13 | 0.75 | 1.60 | 0.15 |
| protein synthesis; tRNA charging | aspartyl/glutamyl-tRNA amidotransferase subunit C | GatC | 0.14 | 0.74 | -0.05 | 0.00 | -0.16 | 0.60 | 0.90 | 0.12 |
| recycling defective proteins | ATP-dependent chaperone/Clp protease | ClpB | 0.91 | 0.11 | 3.02 | 0.01 | -1.06 | 0.16 | 0.48 | 0.29 |
| regulation of catabolic enzyme coding genes | catabolite control protein A | CcpA | 0.27 | 0.15 | 0.74 | 0.01 | -0.16 | 0.31 | 0.07 | 0.68 |
| regulation of sugar uptake PTS system genes | BglG family transcriptional antiterminator | | 0.69 | 0.37 | -1.90 | 0.02 | | . | 0.00 | 1.00 |
| regulatory role (carbohydrate-related) | sorbitol operon regulator | SorC | -1.16 | 0.37 | -2.00 | 0.01 | -0.87 | 0.33 | -0.01 | 0.99 |
| ribosomal RNA processing (LSU) | Ribosomal large subunit pseudouridine synthase B | RluB | -0.84 | 0.11 | -0.05 | 0.00 | 0.95 | 0.36 | -0.37 | 0.38 |
| RNA chaperone | Cold shock-like protein CspLA | CspLA | 0.44 | 0.21 | 0.36 | 0.01 | 0.06 | 0.65 | -0.02 | 0.85 |

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|---|---|---|---|---|---|---|---|---|---|---|
| RNA polymerase | DNA-directed RNA polymerase subunit alpha | RpoA | -0.22 | 0.29 | -0.72 | 0.01 | 0.34 | 0.11 | 0.26 | 0.05 |
| stress regulation? | universal stress protein, UspA superfamily | | -0.02 | 0.95 | -0.05 | 0.00 | -0.77 | 0.17 | 1.15 | 0.09 |
| TCA cycle | pyruvate carboxylase | Pyc | -0.02 | 0.98 | 2.08 | 0.01 | 1.45 | 0.11 | 0.22 | 0.62 |
| tRNA modification | S-adenosylmethionine--tRNA ribosyltransferase-isomerase | QueA | 0.47 | 0.19 | 1.10 | 0.02 | 0.06 | 0.90 | -0.41 | 0.16 |
| tRNA modification | tRNA CCA-pyrophosphorylase | Cca | -1.10 | 0.25 | -1.33 | 0.02 | | . | | . |
| 30S (SSU) ribosome | 30S ribosomal protein S17 | RpsQ | -0.29 | 0.17 | -0.07 | 0.83 | -0.50 | 0.04 | -0.89 | 0.04 |
| 50S (LSU) ribosome | 50S ribosomal protein L35 | RpmI | -0.37 | 0.17 | -0.23 | 0.26 | 2.84 | 0.04 | -3.57 | 0.00 |
| aquisition of amino acids | pyroglutamyl peptidase | Pcp | -0.03 | 0.86 | -0.22 | 0.57 | 1.24 | 0.01 | 0.93 | 0.02 |
| ATP synthesis, H+ extrusion | ATP synthase subunit epsilon | AtpC | -0.90 | 0.33 | -0.70 | 0.20 | -1.89 | 0.01 | 0.51 | 0.04 |
| DNA topological change | DNA gyrase subunit B | GyrA | -0.25 | 0.48 | 0.47 | 0.14 | 3.43 | 0.00 | 1.57 | 0.00 |
| early peptidoglycan biosynthesis | glucosamine-1-phospnate N-acetyltransferase / UDP-N-acetylglucosamine pyrophosphorylase | GlmU | -0.25 | 0.55 | 0.59 | 0.21 | 1.59 | 0.00 | 1.19 | 0.05 |
| fatty acid biosynthesis | acyl carrier protein | AcpP2 | 0.15 | 0.44 | -0.51 | 0.33 | 2.30 | 0.00 | 1.10 | 0.02 |

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|---|---|---|---|---|---|---|---|---|---|---|
| fermentation, end-product formation | formate acetyltransferase | PflB | -1.19 | 0.12 | -0.89 | 0.36 | 1.78 | 0.02 | 2.54 | 0.00 |
| fermentation; end-product formation | acetate kinase | AckA | 0.69 | 0.37 | -0.79 | 0.26 | 1.82 | 0.01 | -1.18 | 0.02 |
| fermentation; end-product formation | L-2-hydroxyisocaproate dehydrogenase | | 0.19 | 0.75 | 0.42 | 0.35 | 2.64 | 0.00 | -0.88 | 0.00 |
| galactose/tagatose catabolism | tagatose 1,6-diphosphate aldolase 2 | LacD2 | 0.18 | 0.32 | -1.49 | 0.13 | 0.83 | 0.01 | 1.64 | 0.02 |
| guanosine/guanine/GMP biosynthesis | GMP synthase | GuaA | 0.03 | 0.89 | 0.03 | 0.93 | 0.34 | 0.03 | 2.06 | 0.00 |
| inositol-related compound degradation | inosose dehydratase | IolE | | . | | . | 1.16 | 0.03 | 1.62 | 0.00 |
| lactose catabolism | Galactose-6-phosphate isomerase subunit lacB | LacB | 1.16 | 0.12 | -1.24 | 0.12 | 1.46 | 0.01 | 0.73 | 0.00 |
| nucleotide excision, mismatch repair | DNA helicase IV | PcrA | -0.69 | 0.50 | | . | 3.14 | 0.00 | 3.09 | 0.00 |
| oligopeptide uptake | oligopeptide ABC-type transporter system permease | OppB2 | 0.69 | 0.37 | | . | -1.73 | 0.02 | 3.09 | 0.00 |
| teichoic acid decoration?, exopolysaccharide biosynthesis? | UTP--glucose-1-phosphate uridylyltransferase | GalU | -0.02 | 0.93 | 0.04 | 0.92 | -3.83 | 0.00 | 1.14 | 0.00 |
| thiamine biosynthesis | Hydroxymethylpyrimidine/ phosphomethylpyrimidine kinase | ThiD2 | 1.16 | 0.12 | | . | 0.69 | 0.05 | 1.41 | 0.05 |
| translation attenuation? | GTP-and nucleic acid-binding protein | EngD | 0.05 | 0.63 | -0.21 | 0.11 | -1.49 | 0.00 | 2.59 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| tRNA modification | tRNA (guanine-N7-)-methyltransferase | TrmB | | . | | . | 1.85 | 0.02 | 1.62 | 0.00 |
| 50S (LSU) ribosome | 50S ribosomal protein L22 | RplV | -0.21 | 0.63 | -0.07 | 0.55 | 0.62 | 0.02 | -0.09 | 0.60 |
| antimicrobial peptide efflux? | antimicrobial peptide ABC-type exporter, ATP-binding/permease components | | 0.69 | 0.37 | | . | -1.98 | 0.02 | 0.36 | 0.65 |
| branched chain amino acid transport | branched-chain amino acid AbC-type transporter, permease component | BrnQ | | . | | . | -1.11 | 0.04 | | . |
| cell division (chromosomal segegration) | chromosome partitioning regulatory protein | ParB | 0.50 | 0.39 | | . | 0.35 | 0.00 | 0.23 | 0.71 |
| cell division; cell shape directing complex | rod shape-determining protein MreB | MreB | 0.17 | 0.28 | -0.67 | 0.09 | 1.06 | 0.00 | 0.07 | 0.55 |
| cell division; divisome complex | cell cycle initiation protein GpsB | GpsB | 0.49 | 0.09 | 0.17 | 0.57 | -0.58 | 0.03 | -0.13 | 0.79 |
| citrate utilization | citrate lyase alpha subunit | CitF | -0.46 | 0.68 | -0.03 | 0.97 | -1.70 | 0.00 | -0.50 | 0.46 |
| citrate utilization | citrate lyase beta subunit | CitE | -0.69 | 0.37 | 0.45 | 0.55 | -1.81 | 0.02 | -0.01 | 0.98 |
| copper export | copper ion translocating P-type ATPase | CopZ | | . | | . | 1.90 | 0.01 | 1.17 | 0.10 |
| dCDP/CDP biosynthesis | cytidylate kinase | Cmk | -0.75 | 0.15 | 1.20 | 0.12 | 1.85 | 0.02 | | . |
| DNA topological change | DNA topoisomerase 4 subunit B | ParE | -0.69 | 0.37 | -1.24 | 0.12 | 1.51 | 0.01 | 1.11 | 0.12 |

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|---|---|---|---|---|---|---|---|---|---|---|
| DNA transformation process | negative regulator of genetic competence; adaptor protein | MecA | -0.01 | 0.99 | -0.41 | 0.31 | 1.57 | 0.00 | 0.37 | 0.38 |
| dTDP biosynthesis | thymidylate kinase | Tmk | -1.19 | 0.12 | -1.61 | 0.16 | -0.11 | 0.00 | | . |
| galactose/tagatose catabolism | tagatose-6-phosphate kinase | LacC | 0.69 | 0.37 | -1.18 | 0.09 | 1.85 | 0.02 | 0.77 | 0.37 |
| glycolysis | fructose-bisphosphate aldolase | Fba | 0.69 | 0.37 | -0.52 | 0.49 | -2.62 | 0.03 | -0.03 | 0.96 |
| glycolysis | aldose 1-epimerase | | 0.58 | 0.26 | | . | -1.49 | 0.00 | 0.14 | 0.64 |
| glycolysis | glucose-6-phosphate isomerase | Pgi | 0.24 | 0.11 | -0.11 | 0.43 | 1.20 | 0.00 | 0.19 | 0.14 |
| glycolysis | dihydroxyacetone kinase, N-terminal domain | DhaK | | . | | . | -1.41 | 0.02 | 0.85 | 0.39 |
| isoprenoid biosynthesis; wall lipid carrier | undecaprenyl pyrophosphate synthase | UppS | -0.92 | 0.10 | -0.75 | 0.37 | 1.85 | 0.02 | -0.69 | 0.22 |
| lactose catabolism | galactose mutarotase | LacX | 0.16 | 0.69 | -0.24 | 0.25 | -0.99 | 0.02 | -0.17 | 0.11 |
| lactose catabolism | galactose-6-phosphate isomerase subunit LacA | LacA | -0.03 | 0.96 | | . | 2.07 | 0.00 | -0.03 | 0.96 |
| late peptidoglycan biosynthesis | UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase | MurE | 1.55 | 0.07 | 1.05 | 0.23 | 1.43 | 0.00 | 0.07 | 0.85 |
| mannose/fructose/sorbose uptake | PTS (Mannose/Fructose/Sorbose family) IIB | LevE/ManX | 0.69 | 0.37 | -0.75 | 0.37 | -1.68 | 0.00 | | . |

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|---|---|---|---|---|---|---|---|---|---|---|
| N-acetylated sugar catabolism | N-acetylated sugar-phosphate isomerase | AgaS | 0.68 | 0.06 | -0.05 | 0.93 | 1.84 | 0.00 | -0.18 | 0.50 |
| NAD(P)H biosynthesis | nicotinate phosphoribosyltransferase | PncB | 1.05 | 0.52 | -0.04 | 0.94 | 2.81 | 0.01 | -0.15 | 0.59 |
| nucleoside salvage; phosphate metabolism | 5-nucleotidase 2,3-cyclic-nucleotide 2-phosphodiesterase | UshA/CpdN | 1.51 | 0.16 | -1.24 | 0.37 | -1.46 | 0.03 | 0.54 | 0.22 |
| pantothenate/CoA biosynthesis | eine decarboxylase/phosphopantothenate--cysteine | CoaBC | | . | -0.03 | 0.97 | 1.57 | 0.00 | | . |
| pentose phosphate pathway | ribulose-phosphate 3-epimerase | Rpe | 2.03 | 0.13 | 0.72 | 0.37 | -1.68 | 0.00 | 0.74 | 0.37 |
| pentose phosphate pathway | 3-hexulose-6-phosphate synthase | | | . | | . | 1.57 | 0.00 | | . |
| peptidase for amino acid acquistion | Zn-dependent metalloprotease, MMP_1 subfamily | | | . | | . | -2.23 | 0.01 | 0.76 | 0.37 |
| phage endolysin | phage N-acetylmuramoyl-L-alanine amidase | | 0.69 | 0.37 | 1.84 | 0.20 | 1.57 | 0.00 | 0.02 | 0.98 |
| protein Cys residue reduction | thioredoxin | TrxA2 | -0.48 | 0.37 | 0.15 | 0.39 | -1.92 | 0.00 | 0.49 | 0.13 |
| protein secretion; SRP pathway (trafficking) | signal recognition particle receptor | FtsY | | . | 0.72 | 0.37 | -1.71 | 0.00 | 1.14 | 0.11 |
| protein synthesis; translation release | ribosome-recycling factor | Frr | -0.37 | 0.12 | 0.24 | 0.47 | 0.30 | 0.02 | 1.16 | 0.07 |
| protein synthesis; tRNA charging | lysine--tRNA ligase | LysS | -0.52 | 0.14 | 0.32 | 0.07 | 1.83 | 0.00 | -0.05 | 0.68 |

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|---|---|---|---|---|---|---|---|---|---|---|
| protein synthesis; tRNA charging | phenylalanine--tRNA ligase subunit beta | PheS | -0.81 | 0.13 | -0.40 | 0.57 | 0.94 | 0.02 | -0.46 | 0.16 |
| protein-methionine sulfoxide reduction | peptide methionine sulfoxide reductase | MsrA | 0.00 | 1.00 | | . | -1.98 | 0.01 | 0.74 | 0.37 |
| PTS general components | phosphoenolpyruvate-protein phosphotransferase | PtsI | 0.32 | 0.17 | -0.24 | 0.14 | 0.79 | 0.00 | 0.11 | 0.27 |
| purine biosynthesis | N5-carboxyaminoimidazole ribonucleotide mutase | PurE | 2.84 | 0.07 | | . | -0.58 | 0.05 | 1.24 | 0.12 |
| purine biosynthesis | phosphoribosylformylglycinamidine synthase 2 | PurL | 0.36 | 0.64 | -0.52 | 0.49 | 3.07 | 0.00 | -0.35 | 0.74 |
| regulation of fructose uptake PTS system genes | fructose uptake PTS operon regulatory protein | | | . | | . | 1.57 | 0.00 | 1.24 | 0.12 |
| regulatory role (unknown) | DNA-binding regulatory protein, YebC/PmpR family | | -0.53 | 0.10 | -0.51 | 0.32 | -1.92 | 0.00 | 0.47 | 0.10 |
| regulatory role unknown | XRE family transcriptional regulator | | -0.06 | 0.72 | 0.31 | 0.17 | -0.62 | 0.02 | 0.38 | 0.16 |
| ribosomal RNA processing (SSU) | ribosomal RNA small subunit methyltransferase A | RsmA | -0.69 | 0.37 | 0.72 | 0.37 | -0.11 | 0.00 | 0.74 | 0.37 |
| ribosomal RNA processing (SSU) | 16S RNA-specific pseudouridine synthase | YtzG | -1.53 | 0.15 | 0.45 | 0.70 | 1.85 | 0.02 | | . |
| ribosome processing (SSU) | ribosome biogenesis GTP-binding protein | Der | -0.40 | 0.39 | -0.05 | 0.93 | 2.30 | 0.03 | | . |
| stress regulation? | universal stress protein, UspA superfamily | | 1.07 | 0.06 | -0.52 | 0.66 | -2.48 | 0.00 | -0.10 | 0.57 |

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|---|---|---|---|---|---|---|---|---|---|---|
| thioredoxin reduction | thioredoxin reductase | TrxB | -0.61 | 0.34 | -0.24 | 0.70 | 2.37 | 0.01 | 0.54 | 0.26 |
| transcription antitermination | transcription antitermination protein NusG | NusG | -1.04 | 0.05 | -0.97 | 0.11 | -1.23 | 0.01 | -0.02 | 0.93 |
| transcription elongation | transcription elongation factor GreA | GreA | -0.23 | 0.22 | -0.22 | 0.42 | -0.42 | 0.02 | 0.21 | 0.23 |
| 30S (SSU) ribosome | 30S ribosomal protein S14 | RpsN | -0.18 | 0.67 | -0.75 | 0.37 | 1.16 | 0.19 | -1.98 | 0.01 |
| 30S (SSU) ribosome | 30S ribosomal protein S1 | RpsA | -0.24 | 0.15 | 0.21 | 0.07 | 0.58 | 0.19 | 0.30 | 0.03 |
| 30S (SSU) ribosome | 30S ribosomal protein S15 | RpsO | -0.30 | 0.18 | -0.61 | 0.08 | 0.19 | 0.39 | -1.40 | 0.01 |
| 30S (SSU) ribosome | 30S ribosomal protein S14 type Z | RpsZ | -0.44 | 0.21 | -0.72 | 0.12 | 0.47 | 0.17 | -0.65 | 0.02 |
| 30S (SSU) ribosome | 30S ribosomal protein S12 | RplL | -1.41 | 0.05 | 0.08 | 0.76 | -0.17 | 0.61 | -1.16 | 0.01 |
| antimicrobial peptide export? | antimicrobial peptide ABC-type exporter, ATP-binding protein | | -0.43 | 0.50 | -1.18 | 0.09 | -0.58 | 0.45 | -1.63 | 0.00 |
| ATP synthesis, H+ extrusion | ATP synthase subunit beta | AtpD | 0.26 | 0.10 | -0.03 | 0.85 | -0.25 | 0.15 | 0.94 | 0.00 |
| cell division; divisome complex | cell division protein FtsA | FtsA | -0.03 | 0.92 | 0.67 | 0.28 | 0.80 | 0.24 | 1.14 | 0.04 |
| cell division; divisome complex | cell division protein FtsZ | FtsZ | -0.11 | 0.52 | 0.11 | 0.16 | -0.09 | 0.18 | 0.63 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| cell division; divisome complex | Z-ring protein bundle promoter, ZapA superfamily | YshA | -0.74 | 0.37 | | . | -0.09 | 0.88 | 1.62 | 0.00 |
| cell wall stress gene regulation? | putatively assoc. with cell wall stress, DUF2154 superfamily | LiaF | | . | | . | -0.79 | 0.37 | -2.34 | 0.01 |
| cell wall stress signal transduction? | cell wall stress sensing sensor histidine kinase | LiaS | 0.69 | 0.37 | | . | -0.81 | 0.37 | -1.63 | 0.00 |
| chemotaxis? | accepting chemotaxis-like domain, DUF948 superfamily | YtxG | -0.03 | 0.89 | -0.58 | 0.45 | 0.26 | 0.74 | 1.62 | 0.00 |
| copper homeostasis | copper homeostasis protein | CutC | -0.20 | 0.45 | -0.50 | 0.24 | 0.34 | 0.12 | -0.29 | 0.02 |
| CTP biosynthesis; nucleoside interconversion | CTP synthase | PyrG | -0.49 | 0.29 | -1.24 | 0.37 | 0.35 | 0.22 | 1.48 | 0.00 |
| deoxynucleotide biosynthesis for DNA | ribonucleoside-diphosphate reductase subunit beta | NrdF | 1.79 | 0.12 | | . | 0.12 | 0.57 | 1.59 | 0.03 |
| dGDP/GDP biosynthesis | guanylate kinase | Gmk | | . | -0.75 | 0.37 | -0.79 | 0.37 | 1.62 | 0.00 |
| DNA repair (base excision) | endonuclease IV(3'-5' exonuclease) | Nfo | 0.69 | 0.37 | -0.02 | 0.88 | 0.27 | 0.73 | -4.45 | 0.00 |
| DNA repair-related | adenine-specific DNA methylase | YtxK | 0.19 | 0.61 | -1.60 | 0.16 | -0.40 | 0.18 | 0.89 | 0.05 |
| DNA replication elongation | DNA polymerase III subunit beta | DnaN | 0.54 | 0.19 | -0.13 | 0.52 | 0.78 | 0.11 | 0.71 | 0.04 |
| DNA topological change | DNA gyrase subunit A | GyrB | 0.17 | 0.69 | -0.53 | 0.23 | -0.39 | 0.17 | 1.67 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| electron transport | NAD(P)H-disulfide dehydrogenase, pyr_redox superfamily | YumB | -1.11 | 0.09 | -1.24 | 0.12 | -1.31 | 0.12 | 3.41 | 0.00 |
| fatty acid biosynthesis | acetyl-CoA carboxylase carboxyl transferase subunit alpha | AccA | 0.69 | 0.37 | 0.72 | 0.37 | 1.34 | 0.25 | 1.28 | 0.03 |
| fatty acid biosynthesis | (3R)-hydroxymyristoyl-ACP dehydratase | FabZ | | . | | . | | . | 2.15 | 0.01 |
| fermentation, end-product formation | L-lactate oxidase | | -0.04 | 0.92 | | . | 0.72 | 0.37 | 1.91 | 0.02 |
| fermentation; end-product formation | D-lactate dehydrogenase, putative | | 0.30 | 0.13 | -0.13 | 0.58 | 0.23 | 0.34 | 0.86 | 0.02 |
| fermentation; end-product formation | alpha-acetolactate decarboxylase | AlsD | -1.19 | 0.12 | -0.03 | 0.97 | 0.80 | 0.17 | 1.11 | 0.02 |
| galactose catabolism | galactokinase | GalK | -0.08 | 0.57 | -1.24 | 0.12 | -0.31 | 0.13 | 2.45 | 0.00 |
| glucose/mannose uptake | PTS(Glucose/Mannose family) IIA | LevD/ManX | 0.49 | 0.06 | -0.60 | 0.09 | -0.15 | 0.75 | -0.95 | 0.01 |
| glutathione biosynthesis | glutathione amide reductase | GarB | 0.70 | 0.06 | -1.24 | 0.12 | 0.59 | 0.15 | 0.78 | 0.04 |
| glycerol catabolism | glycerol kinase | GlpK | | . | | . | | . | -1.63 | 0.00 |
| lactose uptake | PTS(Lactose family) IIA subunit | LacF2 | | . | -0.36 | 0.09 | | . | -2.56 | 0.00 |
| L-aspartate biosynthesis; L-phenylalanine/ L-tyrosine biosynthesis | aspartate aminotransferase | AspC | -0.02 | 0.96 | 0.02 | 0.95 | 0.30 | 0.43 | 3.32 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| late peptidoglycan biosynthesis | UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl- | MurG | -0.73 | 0.42 | -1.60 | 0.16 | -1.05 | 0.16 | 2.06 | 0.02 |
| manganese uptake | manganese transport protein MntH | MntH | | . | | . | 0.80 | 0.32 | -3.68 | 0.00 |
| methylglyoxal removal via acetol | multifunctional methylglyoxal reductase | YvgN | 0.14 | 0.77 | -0.75 | 0.37 | 0.80 | 0.41 | -1.63 | 0.00 |
| methylglyoxal removal via acetol | multifunctional methylglyoxal reductase, putative | | -0.36 | 0.29 | 0.11 | 0.56 | -0.39 | 0.13 | 0.93 | 0.01 |
| NAD(P)H biosynthesis | nicotinate phosphoribosyltransferase | PncB2 | -0.02 | 0.97 | -0.52 | 0.49 | 1.21 | 0.37 | -1.63 | 0.00 |
| NAD(P)H biosynthesis | NH(3)-dependent NAD(+) synthetase | NadE | -0.28 | 0.17 | -0.07 | 0.80 | -0.03 | 0.75 | 0.31 | 0.03 |
| nascent protein folding; protein rescue | chaperone protein DnaK | DnaK | 0.09 | 0.13 | -0.10 | 0.35 | 0.15 | 0.21 | -1.21 | 0.00 |
| nucleoside salvage | promiscuous ribonucleotide (NMP) phosphatase | YutF | 0.41 | 0.48 | -0.44 | 0.56 | 1.11 | 0.12 | -0.35 | 0.01 |
| pentose phosphate pathway | glucose-6-phosphate 1-dehydrogenase | Zwf | -0.03 | 0.90 | 0.39 | 0.16 | -0.06 | 0.84 | 1.19 | 0.00 |
| peptidase for amino acid acquistion | PepP_like Xaa-Pro aminopeptidase, APP_like family | YghT | 0.50 | 0.13 | 0.72 | 0.37 | | . | 2.33 | 0.00 |
| peptidase for amino acid acquistion | oligoendopeptidase F | PepF | 0.15 | 0.37 | -0.21 | 0.68 | -0.30 | 0.06 | 0.81 | 0.01 |
| peptidase for amino acid acquistion | leucyl aminopeptidase S | PepS | 0.00 | 1.00 | | . | 1.25 | 0.13 | 2.70 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| peptidase for amino acid aquisition | D-aminopeptidase dipeptide-binding protein family | DppA | | . | 0.72 | 0.37 | 1.00 | 0.14 | -3.23 | 0.00 |
| phospholipid biosynthesis | glycerol 3-phosphate acyltransferase | PlsX | -0.72 | 0.13 | -0.04 | 0.95 | 0.25 | 0.63 | 1.76 | 0.02 |
| protein Cys residue reduction | thioredoxin family protein | TypP | 0.28 | 0.62 | -0.06 | 0.89 | -0.30 | 0.25 | 0.91 | 0.02 |
| protein Cys residue reduction (NrdDE) | glutaredoxin family protein | NrdH | -1.19 | 0.12 | | . | | . | 2.36 | 0.03 |
| protein synthesis; translation initiation | methionyl-tRNA formyltransferase | Fmt | -0.06 | 0.95 | -0.52 | 0.49 | -0.48 | 0.35 | -1.63 | 0.00 |
| protein synthesis; translation release | peptide chain release factor 1 | PrfA | 0.78 | 0.15 | -1.90 | 0.12 | 0.41 | 0.40 | 0.52 | 0.02 |
| protein synthesis; translation release | peptide chain release factor 3 | PrfC | -1.15 | 0.37 | | . | -0.81 | 0.37 | 1.90 | 0.01 |
| protein synthesis; tRNA charging | histidine--tRNA ligase | HisS | 0.39 | 0.11 | -0.26 | 0.13 | 0.47 | 0.31 | 2.40 | 0.00 |
| protein synthesis; tRNA charging | aspartate--tRNA ligase | AspS | 0.25 | 0.64 | 0.42 | 0.35 | 0.87 | 0.12 | -0.68 | 0.00 |
| protein synthesis; tRNA charging | alanine--tRNA ligase | AlaS | -0.19 | 0.29 | 0.60 | 0.29 | 0.11 | 0.26 | 0.57 | 0.02 |
| protein synthesis; tRNA charging | leucine--tRNA ligase | LeuS | -0.48 | 0.29 | 0.16 | 0.63 | -0.02 | 0.95 | 1.72 | 0.00 |
| purine interconversion/salvage | nucleoside deaminase family protein | | 1.16 | 0.12 | | . | -0.77 | 0.16 | -1.82 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| purine nucleoside interconversion/salvage | purine nucleoside phosphorylase | DeoD | 1.16 | 0.12 | | . | 0.61 | 0.35 | 1.47 | 0.03 |
| recycling defective proteins | Clp endopeptidase ATP-binding subunit | ClpB2 | 0.12 | 0.75 | | . | 0.82 | 0.19 | 1.56 | 0.02 |
| regulation of sugar metabolism | DeoR family transcriptional regulator | | | . | | . | 0.26 | 0.55 | 1.62 | 0.00 |
| regulatory role unknown | PadR family transcriptional regulator | | | . | | . | | . | -1.63 | 0.00 |
| ribosomal RNA processing (SSU/LSU) | ribonuclease III | Rnc | -1.08 | 0.10 | -0.75 | 0.37 | 0.99 | 0.15 | 1.62 | 0.00 |
| RNA degradation (degradosome) | ribonuclease J2 | RnjB | 0.47 | 0.68 | | . | 1.30 | 0.25 | 0.91 | 0.02 |
| RNA polymerase | RNA polymerase sigma factor A | RpoD/SigA | -0.02 | 0.96 | -0.95 | 0.32 | 0.91 | 0.09 | 1.57 | 0.00 |
| sorbose catabolism | L-sorbose 1-phosphate reductase | SorE | | . | -0.75 | 0.37 | | . | -3.13 | 0.02 |
| teichoic acid biosynthesis | glycerolphosphate lipoteichoic acid synthetase | LtsA1 | -0.06 | 0.95 | 0.72 | 0.37 | -0.78 | 0.18 | 1.22 | 0.00 |
| teichoic acid biosynthesis | D-ribitol-5-phosphate cytidylyltransferase | TarI | -0.83 | 0.07 | -3.20 | 0.06 | 0.52 | 0.21 | -0.83 | 0.00 |
| teichoic acid decoration? | glycosyltransferase family protein | | -0.84 | 0.36 | -0.75 | 0.37 | 1.31 | 0.16 | 2.97 | 0.00 |
| transposition | transposase, IS1480 family | | | . | | . | -0.79 | 0.37 | -1.63 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| tRNA modification; thiamine biosynthesis | thiamine biosyn. ATP pyrophosphatase/tRNA sulfurtransferase | ThiI | -0.53 | 0.35 | 0.68 | 0.32 | 1.69 | 0.11 | 2.53 | 0.00 |
| UMP/uridine/uracil biosynthesis | dihydroorotate dehydrogenase A | PyrDA | -0.65 | 0.24 | 0.32 | 0.68 | -0.12 | 0.76 | 1.91 | 0.02 |
| vitamin B6 metabolism | pyridoxine 5'-phosphate oxidase, putative | | 0.12 | 0.65 | | . | 0.00 | 1.00 | 2.80 | 0.00 |
| vitamin B6 metabolism? | pyridoxine 5'-phosphate oxidase-like family protein | | -0.45 | 0.22 | -1.24 | 0.12 | 0.35 | 0.39 | 1.35 | 0.00 |

## Appendix D

## Proteomic Analysis of Lithium Chloride Extracts of *Lactobacillus casei*: Evaluation of Impact of Growth at Low pH on Surface Protein Composition and Bacterial Adhesion to HT-29 Cells

Table A4: Proteomics data of Lithium Chloride extracts *Lactobacillus casei* GCRL163 and MJA12 growth at pH 6.5 and 4.5.

**Spc = Average Spectral Count**
**GC = Lactobacillus casei**
**MJA12 = Lactobacillus casei**

| Protein name | Functional Class | Function | SpC 6.5 GC | SpC 4.5 GC | SpC 6.5 MJA12 | SpC 4.5 MJA12 | Log2 FC GC | *T*- teat GC | Log2 FC MJA12 | *T*- teat MJA12 |
|---|---|---|---|---|---|---|---|---|---|---|
| phosphocarrier protein HPr | Phosphotransferase systems | PTS general components | 377.50 | 179.63 | 370.60 | 142.75 | -1.07 | 0.01 | -1.38 | 0.00 |
| 50S ribosomal protein L30 | Ribosomal proteins | 50S (LSU) ribosome | 96.83 | 10.53 | 137.65 | 19.97 | -3.20 | 0.00 | -2.79 | 0.00 |
| elongation factor Tu | Protein translation (elongation) | protein synthesis; translation | 92.59 | 14.79 | 26.31 | 2.20 | -2.65 | 0.00 | -3.58 | 0.05 |
| DUF1447 superfamily protein | Unknown/uncharacterized | unknown function | 58.16 | 7.11 | 69.79 | 35.71 | -3.03 | 0.00 | -0.97 | 0.01 |
| 30S ribosomal protein S1 | Ribosomal proteins | 30S (SSU) ribosome | 55.19 | 27.28 | 7.23 | 34.78 | -1.02 | 0.05 | 2.27 | 0.04 |
| 30S ribosomal protein S7 | Ribosomal proteins | 30S (SSU) ribosome | 50.86 | 7.49 | 2.18 | 10.61 | -2.76 | 0.00 | 2.28 | 0.04 |
| translation initiation factor IF-1 | Protein translation (initiation) | protein synthesis; translation | 44.14 | 4.21 | 33.78 | 3.51 | -3.39 | 0.00 | -3.27 | 0.02 |
| ABC-type transporter, substrate-binding protein | ABC-type transporter systems | unknown transport | 31.90 | 3.21 | 54.97 | 1.15 | -3.31 | 0.00 | -5.57 | 0.00 |
| hypothetical protein BN194_23630 | Unknown/uncharacterized | unknown function | 28.84 | 271.25 | 56.29 | 202.63 | 3.23 | 0.00 | 1.85 | 0.00 |
| 50S ribosomal protein L27 | Ribosomal proteins | 50S (LSU) ribosome | 26.75 | 0.50 | 12.47 | 0.50 | -5.74 | 0.01 | -4.64 | 0.02 |
| 30S ribosomal protein S21 | Ribosomal proteins | 30S (SSU) ribosome | 24.18 | 0.50 | 37.04 | 0.50 | -5.60 | 0.00 | -6.21 | 0.01 |
| acyl carrier protein | Lipid-related metabolism | fatty acid biosynthesis | 20.11 | 7.50 | 8.50 | 2.82 | -1.42 | 0.01 | -1.59 | 0.00 |
| oligopeptide ABC-type transporter, substrate-binding protein | ABC-type transporter systems | oligopeptide uptake | 18.50 | 7.24 | 39.27 | 1.49 | -1.35 | 0.00 | -4.72 | 0.00 |
| hypothetical protein BN194_24860 | Unknown/uncharacterized | unknown function | 18.21 | 4.53 | 7.62 | 0.50 | -2.01 | 0.01 | -3.93 | 0.01 |
| 50S ribosomal protein L29 | Ribosomal proteins | 50S (LSU) ribosome | 18.14 | 0.50 | 16.64 | 0.85 | -5.18 | 0.00 | -4.30 | 0.00 |
| 30S ribosomal protein S13 | Ribosomal proteins | 30S (SSU) ribosome | 18.11 | 3.54 | 21.17 | 0.50 | -2.36 | 0.01 | -5.40 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| glycine cleavage system H protein | Amino acid-related metabolism | glycine cleavage system | 16.16 | 8.47 | 11.06 | 2.15 | -0.93 | 0.00 | -2.36 | 0.03 |
| RNA-binding protein, S4_2 superfamily | General prediction only | unknown role (DNA replication | 14.54 | 1.50 | 17.54 | 0.83 | -3.28 | 0.01 | -4.39 | 0.00 |
| 30S ribosomal protein S14 type Z | Ribosomal proteins | 30S (SSU) ribosome | 10.51 | 0.50 | 5.54 | 0.50 | -4.39 | 0.00 | -3.47 | 0.01 |
| 50S ribosomal protein L36 | Ribosomal proteins | 50S (LSU) ribosome | 8.51 | 1.15 | 8.11 | 0.50 | -2.89 | 0.00 | -4.02 | 0.00 |
| thioredoxin | Posttranslational modification | protein Cys residue reduction | 8.49 | 1.82 | 3.18 | 1.50 | -2.22 | 0.00 | -1.08 | 0.02 |
| 50S ribosomal protein L33 | Ribosomal proteins | 50S (LSU) ribosome | 6.83 | 0.50 | 10.41 | 0.50 | -3.77 | 0.00 | -4.38 | 0.02 |
| 50S ribosomal protein L32 | Ribosomal proteins | 50S (LSU) ribosome | 6.83 | 3.21 | 5.50 | 0.50 | -1.09 | 0.01 | -3.46 | 0.00 |
| hypothetical protein BN194_15370 | Unknown/uncharacterized | uncharacterised protein | 5.83 | 0.50 | 5.17 | 0.83 | -3.54 | 0.00 | -2.63 | 0.01 |
| ribosome-associated heat shock protein implicated in the recycling of the 50S | tRNA/Ribosome assembly/processing | ribosome recycling (LSU); stress | 5.82 | 3.82 | 3.89 | 0.85 | -0.61 | 0.04 | -2.20 | 0.03 |
| class I heat-shock protein (chaperonin) large subunit | Protein folding/turnover | nascent protein folding | 5.50 | 6.82 | 0.50 | 1.50 | 0.31 | 0.01 | 1.58 | 0.00 |
| DNA-directed RNA polymerase subunit delta | RNA polymerase | RNA polymerase | 5.50 | 10.53 | 0.50 | 9.15 | 0.94 | 0.01 | 4.19 | 0.01 |
| glycine betaine/carnitine/choline ABC-type transporter, substrate- | ABC-type transporter systems | carnitine/choline/glycine betaine uptake | 4.82 | 0.50 | 1.88 | 0.50 | -3.27 | 0.01 | -1.91 | 0.05 |
| triosephosphate isomerase | Central glycolytic/intermediary pathways | glycolysis | 4.50 | 10.50 | 0.50 | 2.49 | 1.22 | 0.00 | 2.31 | 0.02 |
| trigger factor Tig | Protein folding/turnover | nascent protein folding | 3.85 | 23.41 | 1.20 | 10.52 | 2.61 | 0.00 | 3.14 | 0.00 |
| glutamate--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 3.17 | 6.89 | 0.50 | 6.19 | 1.12 | 0.03 | 3.63 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| manganese-dependent inorganic pyrophosphatase | Membrane bioenergetics | ATP synthesis | 3.16 | 11.89 | 0.88 | 4.81 | 1.91 | 0.00 | 2.45 | 0.01 |
| DNA gyrase subunit A | DNA replication-related | DNA topological change | 2.83 | 1.18 | 0.50 | 2.18 | -1.27 | 0.02 | 2.12 | 0.01 |
| von Willebrand factor type A domain protein | Unknown/uncharacterized | unknown function | 2.49 | 8.46 | 4.27 | 0.50 | 1.76 | 0.02 | -3.10 | 0.03 |
| fumarate reductase flavoprotein subunit | Tricarboxylic acid pathway | fermentation; TCA cycle | 2.17 | 0.50 | 3.48 | 0.85 | -2.12 | 0.01 | -2.04 | 0.02 |
| D-lactate dehydrogenase, putative | Carbohydrate-related metabolism | fermentation; end-product formation | 2.17 | 6.18 | 0.50 | 1.83 | 1.51 | 0.00 | 1.88 | 0.02 |
| 50S ribosomal protein L33 | Ribosomal proteins | 50S (LSU) ribosome | 1.83 | 0.50 | 7.33 | 0.50 | -1.87 | 0.01 | -3.87 | 0.02 |
| hypothetical protein BN194_02560 | Unknown/uncharacterized | unknown function | 0.50 | 11.59 | 0.80 | 5.81 | 4.54 | 0.01 | 2.86 | 0.00 |
| aminopeptidase C | Amino acid-related metabolism | peptidase for amino acid acquistion | 0.50 | 3.21 | 0.50 | 2.81 | 2.68 | 0.05 | 2.49 | 0.05 |
| DNA-binding protein HU | DNA replication-related | DNA topology change | 178.88 | 65.34 | 93.50 | 19.56 | -1.45 | 0.00 | -2.26 | 0.08 |
| Cold shock protein 1 | Transcription-associated proteins | RNA chaperone | 67.51 | 23.70 | 41.72 | 53.80 | -1.51 | 0.00 | 0.37 | 0.10 |
| 30S ribosomal protein S16 | Ribosomal proteins | 30S (SSU) ribosome | 58.91 | 11.47 | 24.34 | 7.48 | -2.36 | 0.00 | -1.70 | 0.07 |
| 50S ribosomal protein L7/L12 | Ribosomal proteins | 50S (LSU) ribosome | 54.82 | 37.31 | 29.84 | 18.86 | -0.56 | 0.01 | -0.66 | 0.15 |
| 30S ribosomal protein S19 | Ribosomal proteins | 30S (SSU) ribosome | 54.71 | 5.18 | 44.57 | 7.33 | -3.40 | 0.00 | -2.60 | 0.09 |
| 50S ribosomal protein L16 | Ribosomal proteins | 50S (LSU) ribosome | 45.43 | 5.53 | 18.12 | 4.12 | -3.04 | 0.00 | -2.14 | 0.27 |
| 30S ribosomal protein S10 | Ribosomal proteins | 30S (SSU) ribosome | 44.59 | 5.99 | 0.88 | 0.50 | -2.90 | 0.00 | -0.81 | 0.41 |
| 30S ribosomal protein S8 | Ribosomal proteins | 30S (SSU) ribosome | 43.88 | 6.85 | 5.33 | 7.54 | -2.68 | 0.00 | 0.50 | 0.33 |
| hypothetical protein BN194_25890 | Unknown/uncharacterized | unknown function | 38.47 | 4.50 | 31.84 | 12.23 | -3.10 | 0.00 | -1.38 | 0.06 |
| 30S ribosomal protein S2 | Ribosomal proteins | 30S (SSU) ribosome | 36.22 | 7.79 | 4.09 | 2.86 | -2.22 | 0.00 | -0.52 | 0.63 |

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|---|---|---|---|---|---|---|---|---|---|---|
| DUF1831 superfamily protein | Unknown/uncharacterized | unknown function | 30.48 | 6.50 | 9.03 | 4.50 | -2.23 | 0.00 | -1.00 | 0.10 |
| 50S ribosomal protein L22 | Ribosomal proteins | 50S (LSU) ribosome | 30.48 | 3.53 | 31.23 | 0.85 | -3.11 | 0.00 | -5.21 | 0.06 |
| 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase | Central glycolytic/intermediary pathways | glycolysis | 27.11 | 6.44 | 9.79 | 1.50 | -2.07 | 0.00 | -2.71 | 0.20 |
| hypothetical protein BN194_01830 | Unknown/uncharacterized | unknown function | 24.49 | 1.53 | 10.45 | 3.86 | -4.00 | 0.00 | -1.44 | 0.10 |
| 50S ribosomal protein L15 | Ribosomal proteins | 50S (LSU) ribosome | 24.16 | 9.89 | 1.26 | 0.50 | -1.29 | 0.00 | -1.33 | 0.39 |
| 50S ribosomal protein L13 | Ribosomal proteins | 50S (LSU) ribosome | 23.87 | 6.21 | 3.03 | 3.14 | -1.94 | 0.00 | 0.05 | 0.95 |
| 50S ribosomal protein L17 | Ribosomal proteins | 50S (LSU) ribosome | 23.57 | 2.53 | 3.01 | 0.85 | -3.22 | 0.00 | -1.83 | 0.26 |
| 30S ribosomal protein S4 | Ribosomal proteins | 30S (SSU) ribosome | 16.54 | 2.82 | 1.82 | 1.18 | -2.55 | 0.00 | -0.63 | 0.24 |
| 50S ribosomal protein L10 | Ribosomal proteins | 50S (LSU) ribosome | 15.47 | 32.37 | 1.20 | 1.48 | 1.06 | 0.00 | 0.30 | 0.70 |
| 50S ribosomal protein L18 | Ribosomal proteins | 50S (LSU) ribosome | 14.78 | 3.47 | 15.07 | 3.15 | -2.09 | 0.02 | -2.26 | 0.06 |
| glycerol-3-phosphate ABC transporter substrate-binding protein | ABC-type transporter systems | sugar/glycerol uptake | 12.86 | 1.83 | 1.52 | 0.82 | -2.82 | 0.00 | -0.89 | 0.34 |
| 30S ribosomal protein S17 | Ribosomal proteins | 30S (SSU) ribosome | 12.81 | 1.50 | 1.20 | 0.50 | -3.09 | 0.00 | -1.26 | 0.14 |
| xanthine phosphoribosyltransferase | Nucleic acid/nucleotide metabolism | GMP biosynthesis | 12.50 | 4.47 | 1.20 | 1.18 | -1.48 | 0.00 | -0.02 | 0.97 |
| 50S ribosomal protein L11 | Ribosomal proteins | 50S (LSU) ribosome | 11.87 | 2.50 | 2.20 | 1.14 | -2.25 | 0.01 | -0.94 | 0.24 |
| hypothetical protein BN194_01870 | Unknown/uncharacterized | unknown function | 11.49 | 2.17 | 2.56 | 2.17 | -2.40 | 0.00 | -0.24 | 0.66 |
| 50S ribosomal protein L5 | Ribosomal proteins | 50S (LSU) ribosome | 10.18 | 4.79 | 2.95 | 1.49 | -1.09 | 0.01 | -0.99 | 0.31 |
| L-lactate dehydrogenase | Carbohydrate-related metabolism | fermentation; end-product formation | 9.51 | 44.02 | 3.32 | 8.31 | 2.21 | 0.02 | 1.32 | 0.30 |

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|---|---|---|---|---|---|---|---|---|---|---|
| uracil phosphoribosyltransferase | Nucleic acid/nucleotide metabolism | pyrimidine nucleoside salvage | 8.81 | 3.50 | 1.58 | 1.19 | -1.33 | 0.01 | -0.41 | 0.71 |
| 50S ribosomal protein L4 | Ribosomal proteins | 50S (LSU) ribosome | 8.18 | 0.50 | 0.50 | 0.50 | -4.03 | 0.00 | 0.00 | 1.00 |
| 50S ribosomal protein L21 | Ribosomal proteins | 50S (LSU) ribosome | 7.79 | 0.50 | 0.50 | 0.82 | -3.96 | 0.02 | 0.72 | 0.36 |
| 50S ribosomal protein L9 | Ribosomal proteins | 50S (LSU) ribosome | 7.50 | 1.86 | 1.50 | 1.50 | -2.02 | 0.00 | 0.00 | 1.00 |
| nucleic-acid-binding protein, YlxR family protein | General prediction only | unknown function | 7.50 | 0.50 | 10.49 | 0.85 | -3.91 | 0.00 | -3.63 | 0.10 |
| hypothetical protein BN194_11770 | Unknown/uncharacterized | unknown function | 7.48 | 1.14 | 1.52 | 0.50 | -2.71 | 0.01 | -1.60 | 0.15 |
| XRE family transcriptional regulator | Transcriptional regulation | regulatory role unknown | 7.14 | 0.50 | 4.35 | 2.15 | -3.84 | 0.01 | -1.01 | 0.28 |
| DUF1250 superfamily protein | Unknown/uncharacterized | unknown function | 6.85 | 3.14 | 4.56 | 1.87 | -1.12 | 0.02 | -1.29 | 0.10 |
| hypothetical protein BN194_01080 | Unknown/uncharacterized | unknown function | 6.82 | 2.18 | 3.62 | 0.50 | -1.65 | 0.01 | -2.86 | 0.09 |
| 50S ribosomal protein L19 | Ribosomal proteins | 50S (LSU) ribosome | 6.53 | 1.50 | 0.50 | 0.50 | -2.12 | 0.03 | 0.00 | 1.00 |
| 30S ribosomal protein S15 | Ribosomal proteins | 30S (SSU) ribosome | 6.16 | 1.18 | 0.88 | 1.80 | -2.39 | 0.00 | 1.03 | 0.38 |
| UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) | Cell wall biogenesis | late peptidoglycan biosynthesis | 6.16 | 1.53 | 0.50 | 0.50 | -2.01 | 0.01 | 0.00 | 1.00 |
| 50S ribosomal protein L1 | Ribosomal proteins | 50S (LSU) ribosome | 5.49 | 0.50 | 0.50 | 0.50 | -3.46 | 0.00 | 0.00 | 1.00 |
| 30S ribosomal protein S20 | Ribosomal proteins | 30S (SSU) ribosome | 5.17 | 0.50 | 0.50 | 0.50 | -3.37 | 0.00 | 0.00 | 1.00 |
| phosphonate ABC transporter substrate-binding protein | ABC-type transporter systems | phosphonate uptake | 5.16 | 1.18 | 0.88 | 0.50 | -2.13 | 0.01 | -0.81 | 0.41 |
| ABC-type transporter, substrate-binding protein | ABC-type transporter systems | unknown transport | 5.16 | 0.86 | 2.11 | 1.17 | -2.59 | 0.00 | -0.85 | 0.21 |
| 50S ribosomal protein L14 | Ribosomal proteins | 50S (LSU) ribosome | 5.16 | 0.50 | 0.50 | 0.50 | -3.37 | 0.00 | 0.00 | 1.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| DUF1021 superfamily protein | Unknown/uncharacterized | unknown function | 4.84 | 0.82 | 0.50 | 0.50 | -2.56 | 0.00 | 0.00 | 1.00 |
| DUF2969 superfamily protein | Unknown/uncharacterized | unknown function | 4.83 | 1.82 | 2.44 | 3.83 | -1.40 | 0.00 | 0.65 | 0.17 |
| GTP-binding protein TypA/BipA | Protein translation (elongation) | protein synthesis; ribosome | 4.51 | 1.50 | 0.50 | 0.82 | -1.59 | 0.02 | 0.72 | 0.36 |
| hypothetical protein BN194_24990 | Unknown/uncharacterized | unknown function | 4.51 | 0.50 | 0.50 | 0.50 | -3.17 | 0.00 | 0.00 | 1.00 |
| elongation factor Ts | Protein translation (elongation) | protein synthesis; translation | 4.48 | 10.15 | 1.56 | 0.50 | 1.18 | 0.01 | -1.64 | 0.20 |
| FeS_biosyn superfamily protein | General prediction only | FeS cluster assembly-related? | 4.17 | 0.50 | 0.50 | 0.50 | -3.06 | 0.00 | 0.00 | 1.00 |
| acetyltransferase (GNAT) family protein | General prediction only | unknown function | 4.16 | 1.50 | 0.50 | 0.50 | -1.47 | 0.02 | 0.00 | 1.00 |
| ArsR family transcriptional repressor | Transcriptional regulation | regulation of copper | 3.84 | 0.82 | 0.50 | 0.83 | -2.22 | 0.00 | 0.74 | 0.38 |
| Proline--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 3.84 | 0.82 | 0.80 | 0.85 | -2.22 | 0.02 | 0.07 | 0.93 |
| lipid peroxide thiol-specific peroxidase | Cell defense/detoxification | antioxidant using thioredoxin | 3.83 | 1.53 | 0.50 | 0.50 | -1.32 | 0.03 | 0.00 | 1.00 |
| DUF1827 superfamily protein | Unknown/uncharacterized | unknown function | 3.83 | 0.50 | 0.50 | 0.50 | -2.94 | 0.00 | 0.00 | 1.00 |
| adenylosuccinate synthetase | Nucleic acid/nucleotide metabolism | adenosine/adenine/AMP biosynthesis | 3.82 | 0.50 | 0.88 | 0.50 | -2.93 | 0.01 | -0.81 | 0.41 |
| hypothetical protein BN194_02480 | Unknown/uncharacterized | unknown function | 3.50 | 1.50 | 0.50 | 0.83 | -1.22 | 0.00 | 0.74 | 0.38 |
| DUF1883 superfamily protein | Unknown/uncharacterized | unknown function | 3.50 | 0.50 | 1.64 | 0.50 | -2.81 | 0.00 | -1.71 | 0.39 |
| histidine--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 3.49 | 0.86 | 0.82 | 0.50 | -2.03 | 0.02 | -0.71 | 0.36 |
| hypothetical protein BN194_07900 | Unknown/uncharacterized | unknown function | 3.17 | 0.82 | 1.58 | 1.14 | -1.95 | 0.01 | -0.47 | 0.67 |

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|---|---|---|---|---|---|---|---|---|---|---|
| ribonuclease P protein component | tRNA/Ribosome assembly/processing | tRNA and 4.5S processing | 3.17 | 0.82 | 0.88 | 0.50 | -1.95 | 0.01 | -0.81 | 0.41 |
| Valine--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 3.17 | 0.50 | 1.26 | 1.50 | -2.67 | 0.00 | 0.26 | 0.77 |
| hypothetical protein BN194_29450 | Unknown/uncharacterized | unknown function | 3.16 | 0.50 | 0.50 | 0.50 | -2.66 | 0.02 | 0.00 | 1.00 |
| oxaloacetate decarboxylase, alpha subunit | Tricarboxylic acid pathway | citrate utilization | 3.16 | 0.50 | 0.50 | 0.50 | -2.66 | 0.04 | 0.00 | 1.00 |
| threonine--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 3.16 | 1.50 | 0.88 | 0.82 | -1.08 | 0.01 | -0.10 | 0.92 |
| fatty acid methyltransferase (cyclopropane fatty acid acyl synthase) | Lipid-related metabolism | cyclopropane fatty acid synthesis | 3.16 | 1.50 | 0.50 | 1.15 | -1.08 | 0.01 | 1.21 | 0.11 |
| 50S ribosomal protein L3 | Ribosomal proteins | 50S (LSU) ribosome | 3.15 | 0.50 | 0.50 | 0.85 | -2.66 | 0.04 | 0.76 | 0.39 |
| pyruvate kinase | Central glycolytic/intermediary pathways | glycolysis | 2.84 | 18.18 | 0.82 | 2.51 | 2.68 | 0.00 | 1.62 | 0.07 |
| ribosome-binding factor A | tRNA/Ribosome assembly/processing | ribosome processing (SSU) | 2.84 | 1.50 | 0.50 | 0.50 | -0.92 | 0.02 | 0.00 | 1.00 |
| DUF4330 superfamily protein | Unknown/uncharacterized | unknown function | 2.84 | 0.86 | 1.52 | 0.82 | -1.73 | 0.02 | -0.89 | 0.34 |
| 30S ribosomal protein S11 | Ribosomal proteins | 30S (SSU) ribosome | 2.84 | 0.82 | 0.50 | 0.50 | -1.79 | 0.01 | 0.00 | 1.00 |
| transposase, IS3 family | IS elements/foreign DNA defense | transposition | 2.83 | 1.18 | 1.20 | 0.85 | -1.27 | 0.02 | -0.50 | 0.53 |
| DEAD/DEAH box helicase | tRNA/Ribosome assembly/processing | ribosome processing (LSU); RNA | 2.83 | 0.82 | 0.82 | 0.50 | -1.78 | 0.01 | -0.71 | 0.36 |
| galactokinase | Carbohydrate-related metabolism | galactose catabolism | 2.82 | 0.50 | 0.50 | 0.50 | -2.50 | 0.02 | 0.00 | 1.00 |
| D-ribitol-5-phosphate cytidylyltransferase | Cell wall biogenesis | teichoic acid biosynthesis | 2.50 | 1.50 | 2.21 | 1.50 | -0.74 | 0.00 | -0.56 | 0.62 |

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|---|---|---|---|---|---|---|---|---|---|---|
| ribosome-associated sigma 54 modulation protein | tRNA/Ribosome assembly/processing | ribosome-associated (stabilisation); | 2.50 | 1.50 | 0.50 | 1.18 | -0.74 | 0.00 | 1.24 | 0.13 |
| undecaprenyl pyrophosphate synthase | Lipid-related metabolism | isoprenoid biosynthesis; wall lipid | 2.50 | 1.14 | 0.50 | 0.50 | -1.13 | 0.01 | 0.00 | 1.00 |
| ATP-dependent RNA helicase | tRNA/Ribosome assembly/processing | ribosome processing (LSU) | 2.50 | 0.82 | 1.18 | 1.18 | -1.61 | 0.01 | 0.00 | 1.00 |
| ATP-dependent Clp protease ATP-binding subunit ClpE | Protein folding/turnover | recycling defective proteins | 2.50 | 0.50 | 0.82 | 0.82 | -2.32 | 0.00 | 0.00 | 1.00 |
| hydrolase of HD superfamily | General prediction only | unknown function | 2.50 | 0.50 | 0.50 | 0.83 | -2.32 | 0.00 | 0.74 | 0.38 |
| pyruvate, phosphate dikinase | Central glycolytic/intermediary pathways | glycolysis | 2.50 | 0.50 | 0.50 | 0.50 | -2.32 | 0.00 | 0.00 | 1.00 |
| 23S RNA-specific pseudouridine synthase | tRNA/Ribosome assembly/processing | ribosomal RNA processing (LSU) | 2.50 | 0.50 | 0.50 | 0.50 | -2.32 | 0.00 | 0.00 | 1.00 |
| ATP-dependent DNA helicase | DNA repair/recombination | DNA repair (Uvr excision repair complex; | 2.50 | 0.50 | 0.50 | 0.50 | -2.32 | 0.02 | 0.00 | 1.00 |
| glutamine synthetase transcriptional regulator | Transcriptional regulation | regulation of glutamine | 2.49 | 0.50 | 1.48 | 0.50 | -2.32 | 0.02 | -1.57 | 0.14 |
| hypothetical protein BN194_21380 | Unknown/uncharacterized | unknown function | 2.17 | 0.82 | 1.58 | 1.50 | -1.40 | 0.05 | -0.07 | 0.92 |
| Band_7_flotillin family protein | Unknown/uncharacterized | unknown function | 2.17 | 0.82 | 0.50 | 1.50 | -1.40 | 0.05 | 1.59 | 0.37 |
| exported glycerolphosphate lipoteichoic acid synthetase | Cell wall biogenesis | teichoic acid biosynthesis | 2.17 | 0.50 | 1.18 | 0.50 | -2.12 | 0.01 | -1.24 | 0.14 |
| DUF448 superfamily protein | Unknown/uncharacterized | unknown function | 2.17 | 0.50 | 0.88 | 0.85 | -2.12 | 0.01 | -0.06 | 0.95 |
| UDP-N-acetylmuramoylalanine--D-glutamate ligase | Cell wall biogenesis | late peptidoglycan biosynthesis | 2.17 | 0.82 | 0.88 | 0.85 | -1.40 | 0.04 | -0.06 | 0.95 |
| glutamate 5-kinase | Amino acid-related metabolism | L-proline biosynthesis | 2.17 | 0.50 | 0.50 | 0.50 | -2.12 | 0.01 | 0.00 | 1.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| 50S ribosomal protein L35 | Ribosomal proteins | 50S (LSU) ribosome | 2.17 | 0.50 | 0.50 | 0.50 | -2.12 | 0.01 | 0.00 | 1.00 |
| PTS (Fructose/Mannitol family) IIABC | Phosphotransferase systems | mannitol uptake | 2.16 | 0.50 | 1.18 | 0.50 | -2.11 | 0.01 | -1.24 | 0.14 |
| alanine--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 2.16 | 0.50 | 0.50 | 1.51 | -2.11 | 0.01 | 1.60 | 0.17 |
| ornithine decarboxylase, inducible | Amino acid-related metabolism | polyamine biosynthesis | 2.16 | 0.50 | 0.50 | 0.50 | -2.11 | 0.01 | 0.00 | 1.00 |
| N-acetylglucosamine-6-phosphate deacetylase | Carbohydrate-related metabolism | N-acetylglucosamine catabolism | 2.16 | 0.50 | 0.50 | 0.50 | -2.11 | 0.01 | 0.00 | 1.00 |
| leucine--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 1.84 | 7.21 | 1.20 | 1.85 | 1.97 | 0.01 | 0.62 | 0.29 |
| ATP synthase subunit beta | Membrane bioenergetics | ATP synthesis, H+ extrusion | 1.84 | 7.21 | 1.18 | 1.50 | 1.97 | 0.01 | 0.34 | 0.43 |
| phosphoenolpyruvate-protein phosphotransferase | Phosphotransferase systems | PTS general components | 1.84 | 4.50 | 0.50 | 2.20 | 1.29 | 0.02 | 2.14 | 0.14 |
| ATP-dependent dsDNA exonuclease component | DNA repair/recombination | DNA repair (homologous recombination) | 1.84 | 0.50 | 2.33 | 0.50 | -1.88 | 0.02 | -2.22 | 0.27 |
| glutathione/cysteine ABC-type exporter, ATP-binding/permease | ABC-type transporter systems | glutathione/cysteine export | 1.84 | 0.50 | 0.88 | 0.83 | -1.88 | 0.02 | -0.07 | 0.94 |
| ATP-dependent DNA helicase | DNA repair/recombination | DNA repair (homologous recombination; | 1.84 | 0.50 | 0.50 | 0.83 | -1.88 | 0.02 | 0.74 | 0.38 |
| 3-oxoacyl-[acyl-carrier-protein] reductase 4 | Lipid-related metabolism | fatty acid biosynthesis | 1.84 | 0.50 | 0.50 | 0.50 | -1.88 | 0.02 | 0.00 | 1.00 |
| cadmium efflux system accessory protein | Other transporter proteins | cadmium efflux | 1.84 | 0.50 | 0.50 | 0.50 | -1.88 | 0.02 | 0.00 | 1.00 |
| PTS (Galactitol family) IIB | Phosphotransferase systems | galactitol uptake | 1.84 | 0.50 | 0.50 | 0.50 | -1.88 | 0.02 | 0.00 | 1.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| RNA polymerase sigma factor A | RNA polymerase | RNA polymerase | 1.84 | 0.50 | 0.50 | 0.50 | -1.88 | 0.02 | 0.00 | 1.00 |
| malate dehydrogenase (malic enzyme) | Tricarboxylic acid pathway | fermentation; TCA cycle | 1.83 | 0.50 | 0.50 | 0.85 | -1.87 | 0.02 | 0.76 | 0.39 |
| 3-hydroxy-3-methylglutaryl-coenzyme A reductase | Lipid-related metabolism | mevalonate pathway for isoprenoid | 1.83 | 0.50 | 1.14 | 0.50 | -1.87 | 0.01 | -1.19 | 0.37 |
| transcriptional antiterminator with sigma54 interaction domain | Transcriptional regulation | regulation of sugar uptake PTS system | 1.83 | 0.50 | 0.88 | 0.50 | -1.87 | 0.01 | -0.81 | 0.41 |
| phage protein, DUF4355 superfamily | Prophage genome | phage-associated | 1.83 | 0.50 | 0.80 | 1.18 | -1.87 | 0.01 | 0.55 | 0.45 |
| 6-phosphofructokinase | Central glycolytic/intermediary pathways | glycolysis | 1.51 | 4.89 | 0.50 | 0.50 | 1.69 | 0.05 | 0.00 | 1.00 |
| aldose 1-epimerase | Central glycolytic/intermediary pathways | glycolysis | 1.50 | 4.89 | 0.50 | 1.17 | 1.70 | 0.03 | 1.22 | 0.12 |
| cystathionine beta-lyase PatB | Amino acid-related metabolism | L-methionine biosynthesis | 1.50 | 4.50 | 1.26 | 1.15 | 1.59 | 0.01 | -0.12 | 0.91 |
| ribonucleoside-diphosphate reductase subunit beta | Nucleic acid/nucleotide metabolism | deoxynucleotide biosynthesis for DNA | 1.50 | 2.86 | 0.50 | 0.50 | 0.93 | 0.04 | 0.00 | 1.00 |
| 30S ribosomal protein S18 | Ribosomal proteins | 30S (SSU) ribosome | 1.50 | 2.50 | 0.50 | 1.52 | 0.74 | 0.00 | 1.61 | 0.17 |
| ATP-dependent Clp protease ATP-binding subunit ClpX | Protein folding/turnover | recycling defective proteins | 1.50 | 0.50 | 1.18 | 0.82 | -1.58 | 0.00 | -0.53 | 0.49 |
| D-glycerate dehydrogenase/hydroxypyruvate reductase family protein | General prediction only | unknown function (metabolism of | 1.50 | 0.50 | 0.88 | 1.50 | -1.58 | 0.00 | 0.77 | 0.21 |
| hypothetical protein BN194_01280 | Unknown/uncharacterized | unknown function | 1.50 | 0.50 | 0.80 | 0.85 | -1.58 | 0.00 | 0.07 | 0.93 |
| tagatose-6-phosphate kinase | Carbohydrate-related metabolism | galactose/tagatose catabolism | 1.50 | 0.50 | 0.80 | 0.50 | -1.58 | 0.00 | -0.68 | 0.34 |
| DNA polymerase III subunit gamma/tau | DNA replication-related | DNA replication elongation | 1.50 | 0.50 | 0.80 | 0.50 | -1.58 | 0.00 | -0.68 | 0.34 |

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|---|---|---|---|---|---|---|---|---|---|---|
| tRNA pseudouridine synthase B | tRNA/Ribosome assembly/processing | ribosome processing (SSU) | 1.50 | 0.50 | 0.50 | 1.19 | -1.58 | 0.00 | 1.25 | 0.38 |
| cardiolipin synthase | Lipid-related metabolism | phospholipid biosynthesis | 1.50 | 0.50 | 0.50 | 1.18 | -1.58 | 0.00 | 1.24 | 0.13 |
| 23S RNA-specific pseudouridine synthase | tRNA/Ribosome assembly/processing | ribosomal RNA processing (LSU) | 1.50 | 0.50 | 0.50 | 0.85 | -1.58 | 0.00 | 0.76 | 0.39 |
| polyamine ABC-type transporter, ATP binding protein | ABC-type transporter systems | polyamine uptake | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| HAD-like hydrolase superfamily protein Gph-like | General prediction only | unknown function | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| phosphoribosylaminoimidazole carboxamide formyltransferase/inosine- | Nucleic acid/nucleotide metabolism | purine biosynthesis | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| PTS (ascorbate family) subunit IIB | Phosphotransferase systems | ascorbate uptake | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| peptide methionine sulfoxide reductase | Posttranslational modification | protein-methionine sulfoxide | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| ATP-dependent protease, peptidase subunit | Protein folding/turnover | recycling defective proteins | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| HTH_XRE family transcriptional regulator | Transcriptional regulation | unknown regulation | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| tryptophan--tRNA ligase | tRNA aminoacyl synthesis | protein synthesis; tRNA charging | 1.50 | 0.50 | 0.50 | 0.50 | -1.58 | 0.00 | 0.00 | 1.00 |
| beta-Ala-Xaa dipeptidase | Amino acid-related metabolism | peptidase for amino acid acquistion | 1.17 | 5.14 | 0.50 | 0.50 | 2.14 | 0.00 | 0.00 | 1.00 |
| adenine-specific DNA methylase | DNA repair/recombination | DNA repair-related | 1.16 | 3.18 | 0.82 | 1.15 | 1.45 | 0.02 | 0.50 | 0.49 |
| glucose-6-phosphate 1-dehydrogenase | Central glycolytic/intermediary pathways | pentose phosphate pathway | 0.84 | 3.86 | 0.50 | 0.50 | 2.20 | 0.02 | 0.00 | 1.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| LytR_CspA family transcriptional regulator | Transcriptional regulation | regulatory role unknown | 0.83 | 6.50 | 0.50 | 0.50 | 2.96 | 0.00 | 0.00 | 1.00 |
| hypothetical protein BN194_01760 | Unknown/uncharacterized | unknown function | 0.83 | 2.82 | 0.50 | 0.50 | 1.76 | 0.01 | 0.00 | 1.00 |
| alpha/beta hydrolase family protein | General prediction only | unknown function | 0.83 | 2.14 | 0.50 | 0.50 | 1.37 | 0.04 | 0.00 | 1.00 |
| transcription antitermination protein NusG | Transcription-associated proteins | transcription antitermination | 0.50 | 6.53 | 0.50 | 1.86 | 3.71 | 0.00 | 1.89 | 0.22 |
| phosphate acetyltransferase | Carbohydrate-related metabolism | fermentation, end-product formation | 0.50 | 5.14 | 0.50 | 0.50 | 3.36 | 0.00 | 0.00 | 1.00 |
| GMP synthase | Nucleic acid/nucleotide metabolism | guanosine/guanine/GMP biosynthesis | 0.50 | 4.18 | 0.50 | 0.50 | 3.06 | 0.00 | 0.00 | 1.00 |
| PTS (mannose/fructose/sorbose family) IIB subunit | Phosphotransferase systems | mannose/fructose/sorbose uptake | 0.50 | 3.89 | 0.50 | 0.50 | 2.96 | 0.03 | 0.00 | 1.00 |
| cell wall LPTXG motif anchor domain-containing protein | Cell surface proteins/internalins | unknown function | 0.50 | 3.86 | 1.26 | 0.85 | 2.95 | 0.00 | -0.57 | 0.66 |
| nitroreductase family protein | General prediction only | unknown function | 0.50 | 3.76 | 0.50 | 1.18 | 2.91 | 0.04 | 1.24 | 0.13 |
| L-lactate dehydrogenase, putative | Carbohydrate-related metabolism | fermentation; end-product formation | 0.50 | 3.14 | 0.50 | 0.85 | 2.65 | 0.01 | 0.76 | 0.39 |
| response regulator ArlR | Signal transduction | oxidative stress sensing? | 0.50 | 2.50 | 0.88 | 2.15 | 2.32 | 0.00 | 1.29 | 0.07 |
| repair_PSII superfamily protein | Unknown/uncharacterized | unknown function | 0.50 | 2.47 | 1.12 | 0.50 | 2.30 | 0.02 | -1.17 | 0.09 |
| DNA-binding regulatory protein, YebC/PmpR family | Transcriptional regulation | regulatory role (unknown) | 0.50 | 2.18 | 0.50 | 0.50 | 2.12 | 0.01 | 0.00 | 1.00 |
| beta-N-acetylhexosaminidase | Carbohydrate-related metabolism | polysaccharide degradation | 0.50 | 2.18 | 0.50 | 0.85 | 2.12 | 0.01 | 0.76 | 0.39 |
| Mn/Zn ABC transporter substrate-binding protein | ABC-type transporter systems | Mn/Zn ion uptake | 0.50 | 2.18 | 0.50 | 0.50 | 2.12 | 0.01 | 0.00 | 1.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| peptide chain release factor 1 | Protein translation (peptide release) | protein synthesis; translation | 0.50 | 2.14 | 0.50 | 0.50 | 2.10 | 0.00 | 0.00 | 1.00 |
| ribosome stabilisation protein, Iojap_YbeB family | tRNA/Ribosome assembly/processing | unknown ribosome-associated | 0.50 | 2.14 | 0.50 | 0.50 | 2.10 | 0.00 | 0.00 | 1.00 |
| mevalonate kinase | Lipid-related metabolism | mevalonate pathway for isoprenoid | 0.50 | 1.82 | 0.50 | 0.82 | 1.87 | 0.01 | 0.72 | 0.36 |
| copper homeostasis protein | Cell defense/detoxification | copper homeostasis | 0.50 | 1.82 | 0.50 | 0.50 | 1.87 | 0.01 | 0.00 | 1.00 |
| ATP-dependent Clp protease proteolytic subunit | Protein folding/turnover | recycling defective proteins; | 0.50 | 1.50 | 0.80 | 0.50 | 1.58 | 0.00 | -0.68 | 0.34 |
| apurinic/apyrimidinic 3'-exo-deoxyribonuclease III | DNA repair/recombination | DNA repair (base excision) | 0.50 | 1.50 | 0.50 | 1.15 | 1.58 | 0.00 | 1.21 | 0.11 |
| multidrug ABC-type transporter, permease component | ABC-type transporter systems | toxic substance efflux? | 0.50 | 1.50 | 0.50 | 0.50 | 1.58 | 0.00 | 0.00 | 1.00 |
| UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase | Cell wall biogenesis | late peptidoglycan biosynthesis | 0.50 | 1.50 | 0.50 | 0.50 | 1.58 | 0.00 | 0.00 | 1.00 |
| DNA topoisomerase 1 | DNA replication-related | DNA topological change | 0.50 | 1.50 | 0.50 | 0.50 | 1.58 | 0.00 | 0.00 | 1.00 |
| citrate lyase acyl carrier protein | Tricarboxylic acid pathway | citrate utilization | 0.50 | 1.50 | 0.50 | 0.50 | 1.58 | 0.00 | 0.00 | 1.00 |
| DUF960 superfamily protein | Unknown/uncharacterized | unknown function | 0.50 | 1.50 | 0.50 | 0.50 | 1.58 | 0.00 | 0.00 | 1.00 |
| glyceraldehyde-3-phosphate dehydrogenase | Central glycolytic/intermediary pathways | glycolysis | 51.56 | 44.10 | 8.70 | 42.92 | -0.23 | 0.35 | 2.30 | 0.00 |
| Cold shock-like protein CspLA | Transcription-associated proteins | RNA chaperone | 25.44 | 19.37 | 86.00 | 16.73 | -0.39 | 0.31 | -2.36 | 0.02 |
| tagatose 1,6-diphosphate aldolase 2 | Carbohydrate-related metabolism | galactose/tagatose catabolism | 24.16 | 22.24 | 10.25 | 32.14 | -0.12 | 0.44 | 1.65 | 0.00 |

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|---|---|---|---|---|---|---|---|---|---|---|
| fructose-bisphosphate aldolase | Central glycolytic/intermediary pathways | glycolysis | 16.18 | 18.48 | 0.50 | 5.50 | 0.19 | 0.45 | 3.46 | 0.01 |
| 50S ribosomal protein L24 | Ribosomal proteins | 50S (LSU) ribosome | 10.19 | 7.18 | 3.56 | 0.50 | -0.50 | 0.10 | -2.83 | 0.02 |
| cold shock protein | Transcription-associated proteins | RNA chaperone | 7.77 | 1.14 | 10.06 | 0.50 | -2.76 | 0.12 | -4.33 | 0.02 |
| LytR family cell envelope-related function transcriptional attenuator | Transcriptional regulation | unknown regulation | 5.53 | 7.86 | 2.18 | 0.50 | 0.51 | 0.26 | -2.13 | 0.01 |
| PspC domain-containing protein | Unknown/uncharacterized | unknown function | 3.85 | 1.18 | 0.50 | 1.50 | -1.71 | 0.10 | 1.58 | 0.00 |
| acyl carrier protein | Lipid-related metabolism | fatty acid biosynthesis | 3.18 | 2.53 | 3.50 | 0.50 | -0.33 | 0.54 | -2.81 | 0.00 |
| class I heat-shock protein (chaperonin) small subunit | Protein folding/turnover | nascent protein folding | 3.16 | 3.14 | 0.50 | 2.82 | -0.01 | 0.97 | 2.50 | 0.00 |
| glutamine synthetase | Amino acid-related metabolism | L-glutamine biosynthesis | 2.84 | 3.43 | 0.50 | 1.82 | 0.27 | 0.63 | 1.86 | 0.01 |
| DUF965 superfamily protein | Unknown/uncharacterized | unknown function | 2.83 | 2.50 | 0.50 | 2.18 | -0.18 | 0.36 | 2.12 | 0.01 |
| xylulose-5-phosphate phosphoketolase | Central glycolytic/intermediary pathways | pentose phosphate pathway; D- | 2.49 | 1.78 | 0.50 | 1.85 | -0.48 | 0.70 | 1.88 | 0.02 |
| 30S ribosomal protein S6 | Ribosomal proteins | 30S (SSU) ribosome | 2.49 | 5.54 | 0.50 | 3.81 | 1.15 | 0.18 | 2.93 | 0.02 |
| D-alanine-poly(phosphoribitol) ligase | Cell wall biogenesis | teichoic acid D-alanylation | 2.17 | 1.50 | 3.12 | 0.85 | -0.54 | 0.49 | -1.89 | 0.00 |
| 23S rRNA methyltransferase, SpoU_sub_bind/SpoU_methylase superfamily | tRNA/Ribosome assembly/processing | ribosomal RNA processing (LSU) | 2.16 | 1.86 | 0.50 | 1.85 | -0.22 | 0.58 | 1.88 | 0.02 |
| multifunctional methylglyoxal reductase, putative | Cell defense/detoxification | methylglyoxal removal via acetol | 1.82 | 2.82 | 0.50 | 2.85 | 0.63 | 0.23 | 2.51 | 0.00 |
| CsbD family protein | General prediction only | stress response (phosphate starvation)? | 1.82 | 0.50 | 2.18 | 0.50 | -1.86 | 0.20 | -2.13 | 0.01 |

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|---|---|---|---|---|---|---|---|---|---|---|
| pyruvate carboxylase | Tricarboxylic acid pathway | TCA cycle | 1.52 | 1.47 | 3.20 | 0.83 | -0.05 | 0.97 | -1.94 | 0.02 |
| foldase protein prsA | Protein folding/turnover | protein secretion: exported | 1.50 | 0.50 | 3.18 | 0.50 | -1.59 | 0.16 | -2.67 | 0.00 |
| dTDP-4-dehydrorhamnose 3,5-epimerase | Cell wall biogenesis | teichoic acid decoration | 1.50 | 1.82 | 0.50 | 1.85 | 0.28 | 0.34 | 1.88 | 0.02 |
| PII-type proteinase | Amino acid-related metabolism | protein degradation (casein) | 1.49 | 1.15 | 0.82 | 7.20 | -0.38 | 0.71 | 3.14 | 0.01 |
| peptidoglycan glycosyltransferase | Cell wall biogenesis | cell division; divisome complex; | 1.17 | 1.53 | 2.82 | 0.50 | 0.39 | 0.64 | -2.50 | 0.00 |
| oligopeptide-binding protein OppA | ABC-type transporter systems | oligopeptide uptake | 1.17 | 1.18 | 3.95 | 0.50 | 0.01 | 0.98 | -2.98 | 0.04 |
| hydrolase, HAD superfamily | General prediction only | unknown function | 1.16 | 1.86 | 0.50 | 2.52 | 0.68 | 0.25 | 2.34 | 0.03 |
| phosphoribosylamine--glycine ligase | Nucleic acid/nucleotide metabolism | purine biosynthesis | 1.16 | 0.50 | 1.50 | 0.50 | -1.21 | 0.11 | -1.58 | 0.00 |
| glycerophosphoryl diester phosphodiesterase | Lipid-related metabolism | phospholipid degradation | 0.84 | 0.50 | 1.50 | 0.50 | -0.75 | 0.38 | -1.58 | 0.00 |
| 30S ribosomal protein S12 | Ribosomal proteins | 30S (SSU) ribosome | 0.83 | 8.32 | 0.50 | 6.87 | 3.33 | 0.17 | 3.78 | 0.02 |
| Xaa-Pro dipeptidyl-peptidase | Amino acid-related metabolism | peptidase for amino acid acquistion | 0.83 | 0.82 | 1.50 | 0.50 | -0.01 | 0.99 | -1.58 | 0.00 |
| glutaredoxin family protein | Posttranslational modification | protein Cys residue reduction | 0.50 | 1.18 | 5.52 | 0.83 | 1.24 | 0.13 | -2.73 | 0.00 |
| xylulose kinase | Carbohydrate-related metabolism | xylose catabolism | 0.50 | 0.82 | 1.88 | 0.50 | 0.72 | 0.36 | -1.91 | 0.05 |
| hypothetical protein BN194_07550 | Unknown/uncharacterized | unknown function | 0.50 | 0.50 | 43.16 | 0.85 | 0.00 | 1.00 | -5.67 | 0.00 |
| LemA superfamily protein | Unknown/uncharacterized | unknown function | 0.50 | 0.50 | 2.20 | 0.50 | 0.00 | 1.00 | -2.14 | 0.02 |

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|---|---|---|---|---|---|---|---|---|---|---|
| N-acetylated sugar-phosphate isomerase | Carbohydrate-related metabolism | N-acetylated sugar catabolism | 0.50 | 0.50 | 1.80 | 0.50 | 0.00 | 1.00 | -1.85 | 0.01 |
| cytochrome D ubiquinol oxidase subunit I | Membrane bioenergetics | electron transport | 0.50 | 0.50 | 1.80 | 0.50 | 0.00 | 1.00 | -1.85 | 0.01 |
| cell divisome complex protein | Cytokinesis | cell division; divisome | 0.50 | 0.50 | 1.50 | 0.50 | 0.00 | 1.00 | -1.58 | 0.00 |
| hypothetical protein BN194_25580 | Unknown/uncharacterized | unknown function | 0.50 | 0.50 | 1.50 | 0.50 | 0.00 | 1.00 | -1.58 | 0.00 |
| alpha-crystallin domain heat shock protein | Protein folding/turnover | protein disaggregation (during rapid | 0.50 | 0.50 | 0.50 | 1.50 | 0.00 | 1.00 | 1.58 | 0.00 |